THE BELL–TOADS AND THEIR OPALINID PARASITES[1]

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The Discoglossidae are a well-demarcated family of Anura, including nine Eastern Hemisphere species and one North American representative. The family is evidently decadent. The extant species, with one exception, are sluggish and inactive and some are very restricted in distribution and are veritable hermits in their habits.

The distribution of the Bell-Toads (Discoglossidae)
with the names of their known Opalinids

Liopelma hochstetteri (Fitz.)	southern New Zealand; no Opalinids.
" *hamiltoni* McCulloch	Stephen Is., Central New Zealand, no Opalinids.
Ascaphus truei Stej.	extreme northwestern United States; tadpoles carry *Protoopalina stejnegeri* new species.
Discoglossus pictus Otth.	southwestern Europe, northwestern Africa; *Protoopalina caudata discoglossi* Metcalf.
Alytes obstetricans (Laur.)	Iberian Peninsula and northeastward to Belgium, western Germany and Switzerland; *Cepedea minor* Metcalf.
" *cisternasi* Bosc.	Spain, Portugal; Opalinids never studied.
Bombina bombina (L.)	

[1] The main points of this paper, except the description of *Protoopalina stejnegeri*, were communicated to the American Society of Zoologists and to the Zoological Section of the American Association for the Advancement of Science at their joint session in Washington, Dec. 31, 1924. An abstract of the discussion (Metcalf, 1924, a) was published in *The Anatomical Record*, Vol. 29, No. 2, Dec. 25, 1925.

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["*Bombinator igneus*"] ....Europe (north to Denmark) and east into
                                Asia Minor and Syria; *Protoopalina*
                                intestinalis (Stein.), *P. caudata* (Zel-
                                ler), one report of *Opalina ranarum*
                                (Ehrenberg).
Bombina pachypus (Bonaparte) ....southwestern Europe; *Protoopalina intes-*
                                tinalis (Stein.), *P. caudata* (Zeller).
    "   *orientalis* (Boulenger)......Korea, Manchuria, Shantung, Ussuri,
                                Sakhalin; *Protoopalina orientalis* Met-
                                calf, *P. macrocaudata* Metcalf.
    "   *maxima* (Boulenger) ......southwestern China, northern Tonkin; an
                                undescribed *Protoopalina* closely simi-
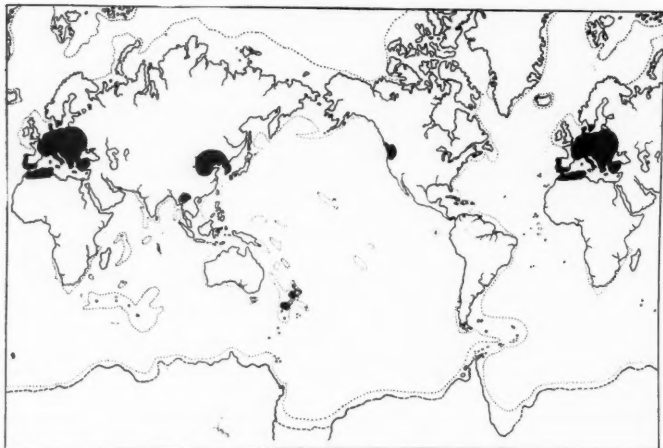   lar to *P. orientalis*.

Fig. 1.   The geographical distribution of the Bell-Toads.

In central and southern Europe and westernmost Asia
are found *Bombina* (2 species) and *Discoglossus pictus,*
the latter extending also into northwestern Africa.   In
western Europe are found *Alytes obstetricans* and *A. cis-*
ternasi.   In eastern Asia occur two more species of
Bombina, one northerly, the other more southerly.   Only
two other genera are known.   These two, though quite
modified, especially in some phases of their life history
and in habitat, are anatomically archaic forms.   They
may well be placed in a subfamily of their own—say the
Liopelminae.   The two genera are *Ascaphus* (1 species)

found in the Olympic Mountains and the northern Sis-
kiyou Mountains and on Mt. Rainier (Tahkoma), and
Liopelma (2 species) from southern and central New
Zealand.

This is a remarkable distribution for a family—western
Euro-Asia and spreading into northern Africa, eastern
Asia, New Zealand and extreme western North America.
It suggests that the family is an ancient and decadent
one. This idea is confirmed by the study of the peculiar
adaptations in the adults in structure, habits and habitat,
by the modified life history in two genera, by the ex-
tremely restricted and peculiar distribution of these two
and by the general unfitness of all the forms for any such
vigorous life as the wide distribution in itself suggests
for the family in the past. Their Opalinid parasites in-
dicate that the family is an ancient one.   Before discuss-
ing them further let us note some features in regard to
each genus.

Alytes, two species. One, *A. obstetricans,* is a sluggish,
retiring, not uncommon species, occurring from Portugal
north to France and east to Switzerland and Germany.
The eggs as laid are taken by the male and fastened upon
his body and hind legs and are carried for about three
weeks; after which he goes to the water, generally in
June, and the larvae hatch out, being at time of hatching
in a rather more advanced stage of development than is
usual for most Anura. This late hatching is correlated
with the large size of the yolk-filled eggs (5 mm. in diam-
eter). The larvae develop slowly, not metamorphosing
until late the next spring, usually in May, but before the
new tadpoles for that season appear in the pools. There
is thus no opportunity for the Opalinid parasites to pass
from tadpole to tadpole throughout the years without the
adults' being infected, as is the case in some Anura whose
tadpoles live more than a year before metamorphosis
(Metcalf, 1924, a). *Alytes cisternasi* is very similar in
structure and habits. It occurs in central Spain and cen-
tral Portugal.

The Opalinid parasites of *Alytes* have not been suffi-
ciently studied.   Collin (1913) reported Opalinid cysts
from *A. obstetricans* from France and I have reported
Cepedea minor from one specimen also from France.
There is no report of any examination of *A. cisternasi* for
Opalinids.   It is quite unexpected to find a *Cepedea* in a
Discoglossid.   Other Bell-Toads carry *Protoopalinae*.   I
should expect more thorough search to disclose similar
Protoopalinae in the two species of *Alytes*.

Bombina bombina ("*Bombinator igneus*") and *B.
pachypus* are closely similar forms, conspicuously marked
on the ventral surface, one species with reddish orange,
the other with lemon yellow.   Both give off a very dis-
agreeable and effective volatile secretion from the skin
when this is pricked.   In my work upon them I have
found that exposure to this secretion produces a choryza
lasting about forty hours.   These are sluggish animals,
easy to catch in the field.   When at rest they are pro-
tectively colored, but when touched they throw up dor-
sally the head and all four limbs, showing the bright
warning color of their ventral surfaces.   Snakes and
turtles will not eat them.   They have the usual Anuran
type of larval development.   Their distribution is similar,
B. pachypus, however, being more to the south and west.

Their Opalinids are two species of *Protoopalina*—
intestinalis and *caudata*—members of two archaic (but
not the most ancient) subgeneric groups of the genus
Protoopalina.

In eastern Asia are two other species of *Bombina*—
orientalis in northeastern China, eastern Russia, Man-
churia, Korea and Sakhalin Island, and *maxima* from the
region eastward of the Himalaya Mountain system.   *B.
orientalis* carries two *Protoopalinae,* very similar to those
in the European Bombinas—*P. orientalis* and *P. macro-
caudata* both belonging to the same subgeneric group as
P. caudata.   *Bombina maxima* bears an undescribed
Protoopalina of which my material is not good enough to
justify describing the species.   It is very similar to *P. ori-*

entalis. In my notes I have been calling it *P. yunnanensis,* and have in several addresses referred to it under this tentative name. The species has, however, never been adequately described and has never been validly named.

Discoglossus pictus, the only species of the genus, is an active little animal, largely aquatic, not more protected than many other Anura by poisonous skin secretions. It occurs in central and eastern Spain, Portugal, Corsica, Sardinia, Sicily, Tunis, Algiers and Morocco. Its Opalinid is a subspecies (*Protoopalina caudata discoglossi*) of a species found in the European Bombinas.

Ascaphus truei, described by Stejneger from the Olympic Mountains in Washington, has since been found on Mt. Rainier (Mt. Tahkoma) and in the Siskiyou Mountains of northern California. It is known from no other places and is found here only in the colder high altitudes in and near the glacial streams, even as they emerge from the ice. Through the kindness of Dr. Stejneger I was able to open a good number of specimens from the United States National Museum but, greatly to my disappointment, all were without Opalinids. Later I learned that in some species of Anura, whose tadpoles do not metamorphose until after more than a year has elapsed, the adults do not carry Opalinids, although the tadpoles are well infected. It then occurred to me that this might be the case in *Ascaphus.* Through the generosity of Dr. Ruthven, of the Ann Arbor Museum, five larvae were obtained, of different ages, from 27 mm. to 49 mm. in length, the latter with the limbs fully developed and exposed but with the tail not yet begun to be absorbed. Each of these was found to be well infected with a new *Protoopalina, stejnegeri,* of which a description is appended. It is very similar to *P. caudata* of the European Bombinas. *Ascaphus,* the only Western Hemisphere Discoglossid, carries the only Western Hemisphere representative of either of the subgeneric groups of *Protoopalina* which are characteristic of Euro-Asian Discoglossids. The indica-

tion of Euro-Asian origin for *Ascaphus* is clear.   The
tadpoles of *Ascaphus* live in the almost ice-cold water of
glacial streams.   The young develop slowly, requiring
nearly two years to reach metamorphosis.   One interest-
ing adaptation in the tadpoles is the huge mouth aiding
them to cling firmly to the rocks in the swift glacial
streams.   Since the tadpoles live over winter and well
into the second summer season as tadpoles, there is
abundant opportunity for one season's larvae to become
infected by Opalinid cysts from the previous season's
larvae, since, as Brumpt showed in 1915, Opalinids in the
recta of tadpoles of numerous species, probably in all,
frequently encyst and pass out with the faeces into the
pools, these cysts, as well as those from the adult Anura,
serving as a source of infection.

The ancestors of *Ascaphus* must have been vigorous
during the Cretaceous period, when they spread from
Euro-Asia, but to-day *Ascaphus* has withdrawn to the
high cold mountains and even in them is of very local
occurrence.   It seems on the verge of extinction.

Liopelma has two species, *hochstetteri* and *hamiltoni*.
The species *hochstetteri* is known only from the moun-
tains of Otago, the southern province of New Zealand.
Here they live along the glacial streams.   Their large,
yolk-filled eggs are laid under stones upon the moist
ground and not in the water, and here they go through
their whole development including metamorphosis, hatch-
ing as little frogs, with, however, a tail.   There is thus
no free larval life.   Without free larvae browsing over
the bottoms of pools there is no opportunity for infection
of this species and this explains the absence of Opalinids
in four specimens of *Liopelma* which through the kind-
ness of Dr. Stejneger I was able to examine, and also in
a number of individuals in New Zealand, which Professor
Wm. Benham, in response to my suggestion, searched for
Opalinids.

The second species of *Liopelma, hamiltoni,* was de-
scribed by McCulloch (1919) from Stephen Island, in

Cook Strait, between the two larger islands of New Zealand. Its breeding habits are not known but its close resemblance to *L. hochstetteri* in structure, and its habit of hiding under stones (Hedley, 1919), make it altogether probable that the life history is similar and that it has no tadpole stage. This is the more probable since in summer there is no water on the island, though in winter there are a few streams. No search for Opalinids has been made in *L. hamiltoni* but in all likelihood it does not carry these parasites.

Failure to find Opalinids in *Ascaphus* was a keen disappointment until search of its tadpoles disclosed their interesting *Protoopalinae,* evidently of Euro-Asian origin. But it was a double disappointment to find no Opalinids in adult *Liopelma* and to know that this genus had no tadpoles to harbor these parasites (Archey, 1922). Even this disappointment, however, is changed to keen interest upon consideration of conditions in Australia.

Raff (1911) has reported *"Protoopalina intestinalis"* in Australia from the recta of two species of *Hyla* and one species of *Limnodynastes,* and a very similar giant species of *Protoopalina* from another species of *Hyla.* I have not seen Raff's forms but am very doubtful of either being true *P. intestinalis;* indeed, I am confident both are different species. But there is no question as to their being *Protoopalinae* of one of the two subgeneric groups characteristic of Euro-Asian Bell-Toads and of *Ascaphus.* A *Protoopalina* of this group in an Australian *Hyla* or Leptodactylid is an anomaly: a Euro-Asian *Protoopalina* in an Australian *Hyla* or Leptodactylid! The Australian Hylas, like the Leptodactylids, are clearly of tropical American origin and neither they nor their ancestors ever were resident in Euro-Asia (Metcalf, 1923 and 1923, a). The Euro-Asian *Hylae* are all so closely related to one another as properly to be described as subspecies of a single species. They have no near relatives in Australia, but are closely similar to one branch of the North American Hylas and they carry *Opalinae*

belonging to a distinctly North American subgeneric group, the *Opalinae angustae* (Metcalf, 1923).

How is it that Australian Hylas and an Australian Leptodactylid, all of originally tropical American origin, bear *Protoopalinae* of Euro-Asian origin? It seems an anomaly. But a little consideration suggests an explanation. The ancestors of the New Zealand *Liopelmae* must have spread from southeastern Asia across Australia to their present home among the cold mountains of southern New Zealand. This must have occurred during the last of the Jurassic period or the earliest Cretaceous, before the Hylas and Leptodactylids arrived in Australasia from tropical America by one or more southern routes. (See Metcalf, 1923 and 1923, a). We can not imagine *Liopelma* itself undertaking any such migration from Euro-Asia. It is too decadent, too closely adapted to a limited and peculiar habitat. Its more vigorous, less specialized, migrating ancestors doubtless had a life history less modified, more like that of other Bell-Toads, with a free-swimming tadpole stage. They would, then, have carried Opalinids, and of the kind characteristic of the Bell-Toads, namely, *Protoopalinae* of the *intestinalis* or *caudata* subgeneric groups. If these ancestral Bell-Toads persisted in Australia until the late Cretaceous and the early Tertiary they would have come into contact with immigrant Hylas and Leptodactylids.

The presence of such *Protoopalinae* in Australia seems, therefore, natural, but not their inhabiting Hylas, and especially Hylas of tropical American origin. This anomaly is resolved when we realize that it is not at all unknown for species of Opalinids, naturally inhabiting Anura of a certain family, to be adopted secondarily by the Anura of another family. The toads (*Bufo*), for example, adopt about all the groups of Opalinids they meet. They are most hospitable and undiscriminating hosts. *Hyla,* on the other hand, is a more exclusive genus. But there are other instances of Hylas accepting and "acclimating" strange Opalinids. The genus *Opalina* is

unknown from South America, the original home of the Hylidae. But when the Hylas passed into North America they met and adopted Opalinas, changing them, to be sure, from the broad to the narrow form, but serving as favorable hosts (Metcalf, 1923). The late adoption of *Opalina* by Hylids is shown by many things, among others by one feature I have not heretofore sufficiently emphasized in this connection: namely, the intergradation between the different forms of *Opalinae angustae* so that species limits are very ill-demarcated.

That the Bell-Toads should have persisted for a geological period in Australia, long enough to come into contact with immigrant Hylas or Leptodactylids, is wholly natural. Indeed, it is not at all unlikely that there may still be living representatives of the family lurking among the little explored mountain groups of the interior. If such be found and if they have aquatic tadpole larvae, I predict that either the adults or the tadpoles will be found to harbor *Protoopalinae* of the *intestinalis* or *caudata* subgeneric groups.

Stejneger, in 1905, discussing the origin and distribution of the Bell-Toads, placed their point of origin at the eastern end of the Himalaya Mountain system, although at that time no Discoglossid was known from that region. Within a month, however, Boulenger described *Bombina maxima* from there.   Stejneger postulated a northward and westward spread for the ancestors of the European *Bombinae,* of *Discoglossus* and of *Alytes,* and a northward and eastward wandering for the forebears of the north China representative, *Bombina orientalis,* and the North American *Ascaphus,* while the ancestors of *Liopelma* took a southeastward course across the East Indies and Australia to New Zealand.   The study of the Opalinid parasites of the Bell-Toads confirms completely Stejneger's conception.   The westward migration to Europe was apparently by a route north of the Himalaya Mountains, the Asia Minor representatives of the family having come down from the Euxine region. Supervening

desert conditions in that portion of Asia north of the great mountain system destroyed the Discoglossids there, leaving the East-Asian *Bombina maxima* and *B. orientalis* isolated. The ancestors of *Ascaphus* apparently spread eastward and then southward along the land strip which during the Cretaceous period stretched to the east along the northern boundary of the Pacific Ocean and then turned south along this ocean's eastern border (Fig. 2).
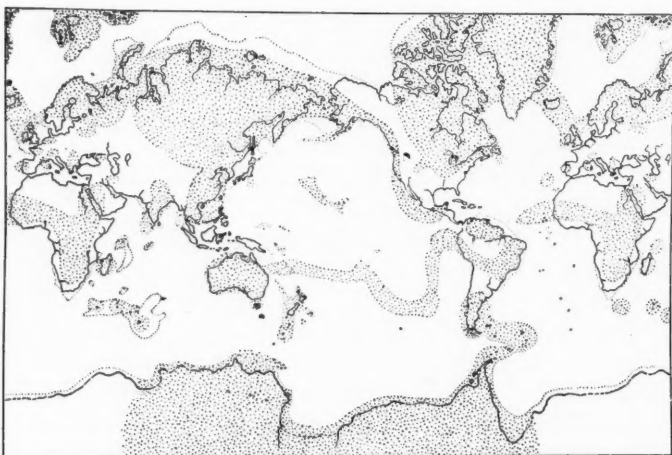

FIG. 2.   Map illustrating Cretaceous continents and the East Pacific Land Strip by way of which the ancestors of *Ascaphus* are thought to have passed to America.

At this time there was a broad ocean channel from the Arctic Ocean to the western part of the Gulf of Mexico so that the ancestors of *Ascaphus* could not pass to the eastward to the North American continent. When, in the early Tertiary, the ocean channel from the Arctic Ocean to the Gulf disappeared and the east Pacific land strip became, in part, fused with the North American continent, *Ascaphus* (or its ancestors), already decadent, remained quiescent, retiring into the cold mountain fastnesses and escaping human observation until after the opening of the "Twentieth Century."

Liopelma has gone still further than *Ascaphus* in its cultivation of the simple life, having even given up the free-living larval stage in its life history. It seems a hopelessly inert species.

The Bell-Toads are in some ways a most instructive group, and their parasites reveal some features of wide interest. The evidence of greater vigor and wide wandering of the earlier Discoglossids, in contrast to the minor place and retiring habit of the modern remnants of the family, is an instructive instance of a phenomenon of wide occurrence, namely, the decadence of once vigorous and dominant forms. Phyla, families, genera, species, like individuals, may come to old age, senescence, decadence, and may thus pass from the scene. The stock may run out just as truly as individuals wear out and the underlying causes may well be in part similar in the two cases. As individuals have a natural length of life and a natural time to give out and die, so some species, genera and major taxonomic groups seem to have as real a time of decadence and death. In individuals, species, genera, families, phyla and all, the cause of senescence, decadence, is doubtless in the stirp, in the germinal stream. Term of life of individual members of a species is a natural character of a species, carried in the germ plasm of the species. A long look over the history of living things upon the earth strongly suggests that a similar natural term of life has been characteristic of many species and genera and of some major groups and may characterize some now living, though not yet shown by their extinction. Length of life, period of persistence, may well be considered partly from the standpoint of the germinal stream and its branches and their inherent potentialities. I hope soon to discuss evolution from the standpoint of the potentialities and trends inherent in the germinal stream and its multitude of branches, emphasizing somewhat more than is customary the internal factors in evolution. The usual discussion of evolution is from the standpoint of individual organisms and their reaction

with the environment. We have missed some suggestive and, I think, informing points of view by thinking not too much of the individual but rather too little of the germinal stream itself upon whose surface the individual is a transient fleck of foam.

One point as to the parasites of the Bell-Toads may well be emphasized. Their distribution indicates that the *intestinalis* subgeneric group of the genus *Protoopalina* arose as early as the Jurassic period, and the structure of the members of the *caudata* subgenus, the persistence of the slender posterior end of the body in one phase of this species (form *acuminata,* Metcalf, 1923), suggests that they are still older. During the whole Cretaceous and Tertiary periods there has, then, been very little modification within these subgenera. Similarly Australian, Patagonian and African representatives of the most archaic subgeneric group of the genus *Protoopalina* have remained almost unmodified. The persistence of parasites for many geologic periods without material modification deserves reiterated emphasis. I have already discussed this (Metcalf, 1923).

The East Pacific Land Strip, of Cretaceous times (Schuchert, 1915; Scharff, 1911), later united with the North American continent and contributed many most interesting species to its extreme western fauna and flora. Among the plants may probably be reckoned the Monterey Cypress, the Monterey Pine, the Torrey Pine and possibly the Sequoias. Among the animals there is perhaps none more interesting than *Ascaphus.* This relict Cretaceous fauna and flora of the islands and coastal mountains of our Pacific coast and of the tip of the peninsula of Lower California are in danger of extinction. Use of some of the islands for pasturing is depopulating them of many of their plants and consequently of some of their animals. Many of these species, both of animals and plants, are no longer vigorous and dominant but are decadent or semidecadent and on the way to natural extinction. They have not the plasticity

to enable them to withstand changing conditions. A prompt and thoroughgoing survey of the regions bearing this relict fauna and flora is immediately important. It should include not only the islands and coastal mountains of the Pacific coast of North America but also the tip of Lower California, the mountain ranges of Middle Central America which have an easterly-westerly trend, the Galapagos Islands and Ecuador, all of which regions show indication of former connection with the ancient East Pacific Land Strip. The Samoan and some other mid-Pacific islands should also be brought into consideration.

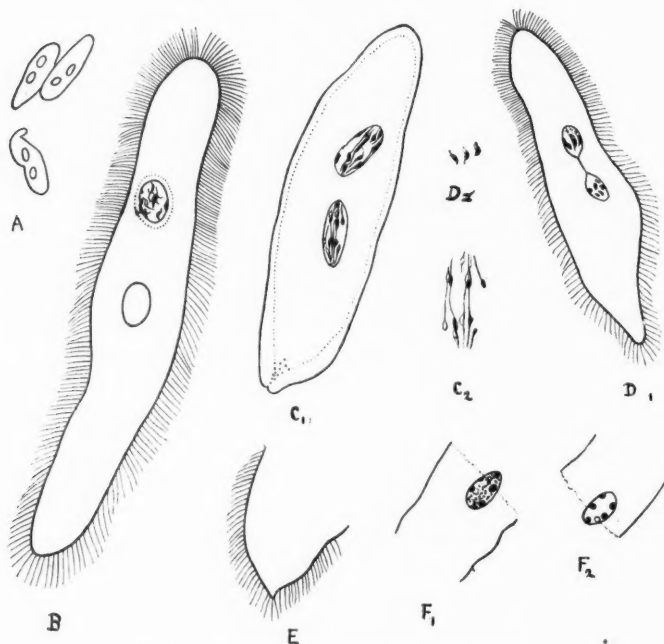### DESCRIPTION OF *Protoopalina stejnegeri*, NEW SPECIES

Host *Ascaphus truei* Stejneger, tadpoles only, adults not infected. Locality, Laundry Creek near Lake Cushman, among the Olympic Mountains, western Washington, U. S. A. Five tadpoles examined, all well infected: *A*, University of Michigan, Number 54308, 25 mm. long, June 25, 1919; *B*, Number 54302, 33 mm., Aug. 5, 1919; *C*, Number 54302, 40 mm.; *D*, Number 54308, 47 mm., June.25, 1919, hind legs half emerged; *E*, Number 54302, 49 mm., legs fully formed and exposed, tail not yet begun to be absorbed, intestine much reduced, only one and a half whorls in the spiral,[2] all collected by Mrs. H. T. Gaige.

MEASUREMENTS IN MILLIMETERS OF PROTOOPALINA STEJNEGERI

	Large	Medium	Small
Length of body	0.17	0.124	0.0625
Width of body	0.024	0.023	0.017
Length of nucleus	0.014	0.013	0.007
Width of nucleus	0.0095	0.0082	0.0065
Length of endospherules	0.0095	0.0015	0.0065
Width of "		0.002	
Thickness of "		0,0015	

This species is named for Leonhard Stejneger, Curator of Vertebrates in the United States National Museum,

[2] This persistence of infection well into the period of metamorphosis seems not exceptional. In *Hyla versicolor* full grown adults are generally not infected, though even the oldest tadpoles are always so, so far as my thirty or more examinations have shown.

FIG. 3. *Protoopalina stejnegeri*, new species

A. Three individuals from tadpole "E" (see text), magnified about 50
       diameters.
B. From tadpole "D"; magnification about 450 diameters.
C_1 An individual from tadpole "D," in the condition in which fission
       usually occurs. Note the indication of the excretory pore at the
       posterior end. Magnification about 450 diameters.
C_2 The macrochromosomes and their chromatin spindle fibers in the pos-
       terior nucleus of the individual shown in C_1. Magnification about
       900 diameters.
D_1 A daughter cell from tadpole "D" not long after completion of fission.
       Magnification about 450 diameters.
D_2 The six macrochromosomes from the anterior nucleus in the last figure;
       those on the upper surface black, those at a deeper level stippled.
       Magnification about 450 diameters.
E. The posterior end of an individual from tadpole "D" showing the
       posterior point. One of the individuals in drawing A shows a pos-
       terior point of the *Protoopalina caudata* type. Magnification about
       450 diameters.
F_1 and F_2 the posterior and the anterior fragment of a broken individual
       from tadpole "D," showing the six macrochromosomes, in each
       nucleus. Magnification about 400 diameters.

who first described *Ascaphus*. The author is deeply in-
debted to him for the privilege of searching the National
Museum Anura for Opalinid parasites and for repeated
assistance in the course of eight years' work upon these
Anuran parasites.

This species is somewhat intermediate in form of body
between *Protoopalina orientalis* from Korea and Man-
churia and *P. intestinalis* of Europe, except that in some
individuals it has a ''tail'' something like that of *P. cau-
data* or *P. macrocaudata,* though usually much less devel-
oped. The cilia are unusually long, in some individuals
0.01 mm. to 0.012 mm., and may be so in all. Cilia are
usually not well preserved when the Opalinids are killed
and preserved within the host. The shorter cilia of some
individuals may be due to poor preservation. The cilia
cover the whole body, there being no naked posterior end,
such as there is in *P. orientalis.* No slender forms like
P. caudata acuminata have been seen.

The nuclei are sometimes connected by a thread (Fig.
3, D) but usually they are completely separated. The
proportion of individuals with connected nuclei is much
smaller in *P. stejnegeri* than in *P. orientalis* and is less
than in *P. caudata, P. intestinalis* and *P. macrocaudata.*
There are six macrochromosomes.

In some full-grown cells of *P. stejnegeri* the nuclei,
after they become separated from each other and are no
longer connected by a thread, show a more diffuse reticu-
lated condition of the chromatin than is usual among
binucleated Opalinids (Fig. 3, B). Individuals in fission
have not been observed, but study of a large series of
adults and daughter individuals indicates that fission
occurs while each of the two nuclei is in an anaphase of
mitosis and before it becomes dumb-bell-shaped. The
nuclei of *P. caudata,* in contrast, are usually already con-
stricted and dumb-bell-shaped when fission occurs.

Protoopalina stejnegeri is intermediate in character
between *P. caudata* and *P. intestinalis* and indicates that

my subgeneric groups "3"[3] and "4" (Metcalf, 1923) are close relatives. *P. stejnegeri* should be placed in Group 3.

The New Species of Protoopalina from *Bombina maxima*

This species I am not attempting to describe from my inadequate material, and so of course I am not giving it a name. It is quite similar to *P. orientalis* though distinct. It was seen in one scant and poorly preserved infection of *Bombina maxima* (Boulenger) from United States National Museum specimen number 68068, 53 mm. long, a female with immature eggs. Collected by Wulsin, May 1st, 1924, at Yen Yen Sung, Indo-China, on top of pass between the Red and the Black Rivers, near the Chinese frontier.

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[3] Subgeneric group 3 includes the species *caudata, macrocaudata, orientalis*, with *stevensoni, australis* and *stejnegeri*: Group 4 includes the species *intestinalis, pelobatidis* and *hylarum*.

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my subgeneric groups "3"[3] and "4" (Metcalf, 1923) are close relatives. *P. stejnegeri* should be placed in Group 3.

The New Species of Protoopalina from *Bombina maxima*

This species I am not attempting to describe from my inadequate material, and so of course I am not giving it a name. It is quite similar to *P. orientalis* though distinct. It was seen in one scant and poorly preserved infection of *Bombina maxima* (Boulenger) from United States National Museum specimen number 68068, 53 mm. long, a female with immature eggs. Collected by Wulsin, May 1st, 1924, at Yen Yen Sung, Indo-China, on top of pass between the Red and the Black Rivers, near the Chinese frontier.

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TWO EXTREME "SMALL" MATTHIOLA PLANTS: A HAPLOID WITH ONE AND A DIPLOID WITH TWO ADDITIONAL CHROMOSOME FRAGMENTS[1]

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A WHITE-FLOWERED, glabrous, double-throwing variety of stock (*Matthiola incana* R. Br.) known as "Snowflake" produces frequent mutants, including at least eight distinct types. These forms are simple trisomics, with fifteen somatic chromosomes; their progeny are always partly normals with fourteen chromosomes. At Ithaca, N. Y., normal parents produced about 2 to 5 per cent. of mutants, the percentage being higher with good germination (Frost, 1919). Among 2,072 progeny of parents presumably normal in chromosome number,[3] giving about 5 per cent. of mutants, nine plants (0.43 per cent.) belonged to a type which has been called Small-smooth-leaved. On account of its strikingly small, flat leaves, this type was very distinct and easily recognized (Frost, 1919, p. 144, Fig. 25). At Riverside, among several thousand Snowflake plants, very few mutants have been referred, even doubtfully, to this type, and it may

[1] Paper No. 159, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] The cytological evidence presented in this paper was mainly obtained by the senior author (of the Division of Genetics, University of California); the evidence on somatic characters was obtained by the junior author (of the Citrus Experiment Station).

[3] Of the nine Small-smooth plants, seven were progeny of Early parents, which gave 956 of the 2,072 total progeny. The Early type has been lost, and its chromosome number has not been determined, but it was discovered only once and probably originated by gene mutation (Frost, 1919). It did, however, give a slightly higher total percentage of chromosomal mutants among its progeny than did the normal Snowflake parents.
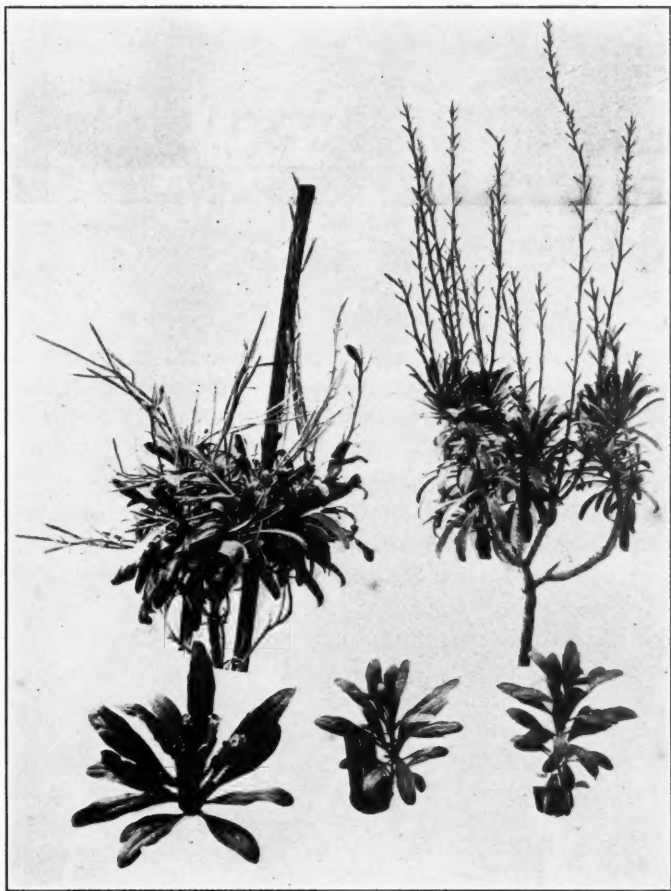
Fig. 1. Greenhouse plants, sibs of those shown in text figures 2 and 3. Upper row: at left, normal; at right, Small. Lower row, from left to right: normal, Small, Small. Note the numerous small leaves, elongated leafy stems and sterile racemes of Small.

have been less common than in the Ithaca cultures mentioned.[3] The failure of the Snowflake Small-smooth plants to produce seed has limited the observations to the occasional mutants, and none of these have been examined cytologically.

FIG. 2. Field plants. At left, extreme Small (25.25–61, tetrasomic diploid); at right, normal. Final development, the Small plant dying (July 1, 1926).

In 1925, however, seed was secured from a hybrid individual which was similar to the earlier non-hybrid Small-smooth-leaved plants. This hybrid mutant occurred in an F_1 family from a cross between a Snowflake trisomic (Large) and a normal plant of a compact, glabrous, deep-red, pure single race obtained from Miss Edith R. Saunders. The hybrid mutant and its mutant-type progeny have resembled the Small-smooth-leaved mutants derived from Snowflake and may represent the same trisomic form. In any case the name ''Small'' is strikingly appropriate to the hybrid type, and the double name ''Small-smooth'' can be retained for the older mutants until the identity of the two forms is established.

The following description of the Small trisomic is based on the hybrid mutant (F_1) and its F_2 progeny (text Figs. 1 and 3). In comparison with the normal (diploid) type from the same cross, the leaves are comparatively numerous, small, flat and rigid, and the corolla and capsules are usually conspicuously reduced in size. There is also a greater tendency toward dentation and sinuation of the leaf margin. Seed production is much less abundant than with normals, and complete sterility may be produced by growth conditions under which normals are

fairly fertile (text Fig. 1). The usual time of flowering seems about the same as with normals, and the average size of plant seems less reduced than with two other trisomics, Crenate and Dark; in these respects the hybrid Small plants appear to disagree with the published description of the Small-smooth mutants observed at Ithaca (Frost, 1919, p. 144).

Seed from guarded pollination of the F_1 Small mutant was sown in 1925 and 1926; the progeny were partly of the parental type and partly normal. Of the Small progeny, ten were examined cytologically, and their pollen mother cells were found to contain a very small unpaired chromosome in addition to the normal seven pairs. This chromosome is much smaller than any of the normal chromosomes of *Matthiola,* and is therefore inferred to be a fragment of a normal chromosome.

At the first or reductional division the unpaired fragment either divides or segregates. If it divides at the
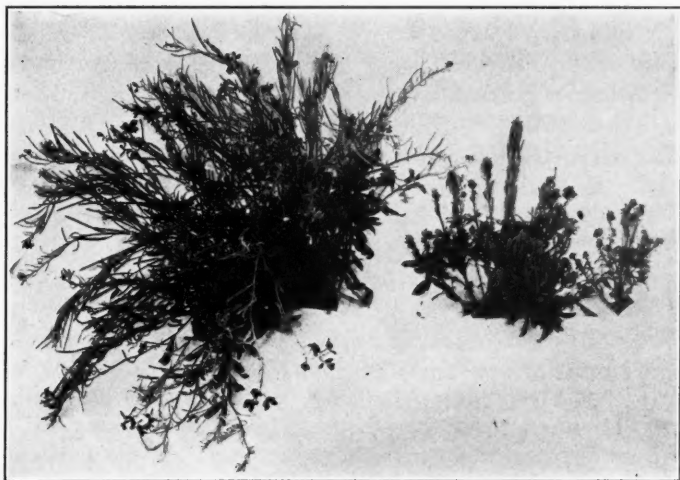

Fig. 3. Field plants. From left to right: normal, trisomic Small, extreme Small (25.25–42, disomic haploid). Late in flowering season (Mar. 18, 1926); the racemes of the haploid made some further growth. The difference between the normal and trisomic plants may be partly due to a recessive compactness character which is not shown by the normal.

first division it segregates at the second, and *vice versa;* since it usually lags behind the paired chromosomes its behavior is very easily seen.

It has already been noted (Frost and Mann, 1924) that some of the pollen mother cells of "Snowflake" *Matthiola* plants possessing the normal number of chromosomes show certain irregularities in cytological behavior— enough to account for the percentage of chromosomal mutants which appears among their progeny. These irregularities include non-disjunction, non-conjunction, and, most commonly of all, fragmentation. In the Small type the small odd chromosome must have resulted from fragmentation.

Many of the F_2 Small trisomics are conspicuously un- like normals, but some difficulty has been encountered in classification, especially with the 1925 progeny. Careful study of the 1926 culture while most plants were in early bloom, however, resulted in unquestioned classification of fifty-five plants out of a total of sixty produced by a sowing of sixty-five seeds; this classification was con- firmed by chromosome counts for seven plants (four Small and three normal) representing most of the prin- cipal F_2 segregant types. Cytological examination of the five doubtful individuals (three of these in poor condi- tion) showed that these were all normals, although three had been thought to be Small. The totals were: Twenty- eight normal, thirty-one trisomic Small and one extreme (tetrasomic) Small. The less positive totals for the 1925 progeny (ninety-four plants from 104 seeds) were: Fifty-six normal, thirty-six trisomic Small and two un- questioned extreme Small; the somatic classification of trisomic Small was confirmed by cytological examination in five cases, while a sixth plant found to have the little extra chromosome had been recorded as normal but was later questioned. The difficulty in classification has been due mainly to the following circumstances: (1) The dif- ferences between normal and Small are very largely in size of parts; (2) some plants were in poor condition

when examined; (3) F_2 segregation had occurred for at least one recessive gene which affects the size and the dentation of the leaves.

As noted above, two of the 1925 F_2 plants showed the Small characteristics in an extreme degree. Text Figs. 2 and 3 show the two "extreme" plants, in comparison with ordinary Small and with normal. The extreme plant in Fig. 2 was very small and compact, with very small leaves, and apparently none of its flower buds ever opened. The extreme individual in Fig. 3 was somewhat larger and showed considerably more development of branches, but its leaves were very small and the few flowers which opened had minute, rudimentary petals. Both plants therefore showed an intensification of the peculiarities of ordinary trisomic Small. The difference in growth habit was probably due to the heterozygosis of the F_1 parent for genes affecting compactness of plant form.

Both of these plants were thought to be simple tetrasomics (if one may use that term for quadruplication of a portion of a chromosome), since they both, like the other tetrasomics which have been studied cytologically (in *Matthiola,* extreme Large, Slender and Smooth), showed an accentuation of the characters of the trisomic type. Upon cytological examination, however, it was found that while one of these plants (25.25–61, text Fig. 2) was a tetrasomic diploid ($7_{II} + 2$), the other (25.25–42, text Fig. 3) was a disomic haploid ($7_I + 1$). It seems fair to conclude that the similarity of these two plants was due to the fact that the balance between whole chromosomes and the fragment or fragments was the same in both, the haploid containing one set of 7 plus 1 fragment, and the tetrasomic, two sets of 7 plus 2 fragments. This conclusion is strengthened by the fact that the tetrasomic Small plant in the cultures of 1926 was of open growth habit, like the haploid, and resembled the haploid plant much more closely than did the compact tetrasomic Small of the 1925 cultures.

The pollen mother cells of the haploid were about one half as large as those of the trisomic (diploid) Small plants. Measurements of drawings of pollen mother cells at the first metaphase (magnification 4000 ×) gave the following average diameters of the drawings: 63.5 mm for six pollen mother cells of trisomic Small and 53 mm for three pollen mother cells of the haploid. The relative volumes were thus approximately 256,048 to 148,877 or nearly 2 : 1. A glance at plate I, Figs. 1b and 2f, will show that when *four microspores* are formed from a pollen mother cell in the haploid plant each microspore is approximately *half* the size of those in the tetrads of trisomic Small, while when *dyads* are formed in the haploid each of the two microspores is about of the *same* size as the microspores in the tetrads of trisomic Small and about *half* the size of the microspores in the dyads of the latter.

The chromosomes of the haploid in first metaphase look like those which one finds at the second metaphase of ordinary diploid or trisomic diploid *Matthiola* plants. (Compare Pl. I, Figs. 1a and 2a.) They resemble second-metaphase chromosomes in size and shape as well as in their position on the spindle. In all these respects they differ radically from the paired first-metaphase chromosomes of diploid and tri- or tetrasomic plants. (In Pl. I, compare Figs. 2a and 2b with Fig. 3a.) The chromosomes in the haploid usually form a flat plate and all divide with the occasional exception of the fragment (Pl. I, Fig. 2c), giving rise to anaphase figures which resemble those of the second anaphase in diploid pollen mother cells. Following such a division a dyad is formed. Sometimes, however, one or a few chromosomes may segregate and the rest divide, and occasionally all chromosomes may segregate, giving rise to pictures like Fig. 2d, which shows three whole chromosomes and the fragment at one pole and four whole chromosomes at the other pole. Such whole chromosomes may divide later and tetrads then result. Obviously, since the segregation is random, many
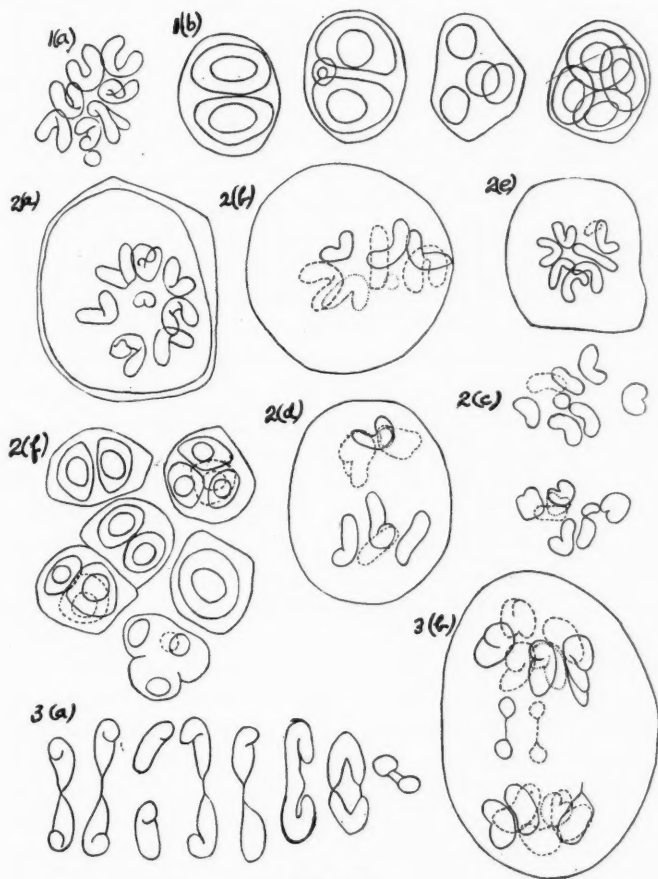
PLATE I

All the chromosome groups, except 2e, represent pollen mother cells. Figure 2e shows a somatic cell from a young bud. The chromosomes were drawn at a magnification of 4,000 diameters, and the tetrads at 1,300 diameters. Drawings reduced approximately 1/3 in reproduction.

FIG. 1. (a) Polar view of second metaphase of trisomic Small. (b) Tetrads and dyads from trisomic Small.

FIG. 2. (a) Polar view of first metaphase of haploid Small (plant 22.25–42). (b) Lateral view of same for comparison with lateral views of first-metaphase chromosomes in tetrasomic Small. (c) Lateral view of first anaphase (non-reductional) of haploid Small. (d) First anaphase following segregation of chromosomes in same. (e) Somatic metaphase of haploid Small. (f) Group of monad, dyads, triad and tetrads of the same.

FIG. 3. (a) Lateral first metaphase of tetrasomic Small (plant 25.25–61). The two fragments are paired. The chromosomes are drawn in series for convenience. (b) Lateral first anaphase of same, showing two separate fragments dividing later than the paired chromosomes.

of the tetrads would be expected to have two pairs of microspores, differing more or less in cell and nuclear size. Of 406 groups of microspores after cytokinesis, 285 or 70 per cent. were dyads with cells of equal size, ninety-nine were triads with the three cells usually very unequal in size, and only nineteen were tetrads—and in these also the microspores tended to be very unequal in size. Three monads were seen; in these the nuclei were irregularly lobed so that it seems probable that they were the result of inhibited division. We may conclude then that, in the *Matthiola* haploid, non-reduction had occurred in about 70 per cent. of the pollen mother cells, while only 30 per cent. had undergone partial or complete reduction. The pollen grains differ considerably in size but most of them are about as large as those found in trisomic Small plants. Unfortunately the haploid plant is dead, so that its pollen can not be tested genetically.

A few chromosome counts were made from somatic cells of young buds of the haploid plant. In these cells, seven large and one small chromosome were clearly seen (Pl. I, Fig. 2e).

The other of the two extreme Small plants described (25.25–61) was a simple tetrasomic, containing two of the chromosome fragments in addition to the normal seven pairs (Pl. I, Figs. 3a and 3b). In some but not all cases these two extra elements were paired at the first metaphase. As a rule reduction occurred, with the consequent production of four equal microspores. About one per cent. of dyads (five out of 487) was found, and about the same number of triads. The pollen mother cells, tetrads and pollen grains were very much like those of trisomic Small in size. After this paper was completed for publication, cytological preparations were secured from the extreme Small plant in the cultures of 1926. This plant is a tetrasomic diploid, and most of its pollen tetrads appear normal.

Haploid plants have been described in only two other genera, several in *Datura* (Blakeslee *et al.,* 1922) and two

in *Nicotiana* (Clausen and Mann, 1924).[4]   In all these cases the haploid was found as a weak maternal type in F_1 populations.   Consequently it is evident that these haploids are, to quote Davenport (1923), "probably due to a process of true parthenogenesis of the reduced egg." Unfortunately, the *Matthiola* haploid occurred in an F_2 population, and therefore its somatic characters throw no light on its mode of origin.   In the *Matthiola* haploid the chromosomes usually formed normal-looking meta-phase plates of the second-division type, and non-reduction occurred in 70 per cent. of the pollen mother cells, while complete reduction occurred only rarely.   In both *Datura* and *Nicotiana* haploids, on the other hand, most of the chromosomes tend to segregate in spite of their unpaired condition, and they much less frequently form a flat, normal-looking metaphase plate resulting in non-reduction.   In the haploid *Datura* and *Nicotiana* (Chipman and Goodspeed, 1927) no gamete containing less than the haploid number of chromosomes is viable.

All the *Datura* trisomics "are of feebler growth than normal and have a high degree of pollen sterility" (Blakeslee *et al.,* 1920).   The tetrasomic plants are smaller and weaker as well as less fertile than the trisomics, and they show considerable exaggeration of the somatic characters peculiar to the corresponding trisomic types (Blakeslee, 1922).   It is considered that the addition of one univalent chromosome to the normal complex unbalances the organism in a certain direction, while two extra chromosomes of the same type cause these characters to be intensified.   This is generally true also of the *Matthiola* trisomics and tetrasomics, including Small, in which only a fragment is triplicated or quadruplicated.

Bridges (1922 and 1925) has shown that the sexual condition in *Drosophila* is a function of the balance between

4 Gaines and Aase (*Am. Jour. Bot.* 13: 373–385) have recently described a haploid wheat plant whose cytological behavior resembles that of the *Nicotiana* and *Datura* haploids.   Like those forms it was maternal in type and almost completely sterile.   In vigor, however, it was not inferior to the diploid maternal race.

X-chromosomes and autosomes. He suggests that the variation in sexual characters which accompanies variation in the numerical ratio between X-chromosomes and autosomes is due to the different internal balance of these two kinds of chromosomes with respect to genes affecting sex characters. This hypothesis was originally based largely on the fact that haploidy and triploidy for the small fourth chromosome produce opposite effects on many characters, indicating that this chromosome has a different genic balance from the others. This conception has made it possible for Bridges to predict the sex type to be expected from various combinations of chromosomes. One of the most interesting cases in which such a prediction has proven correct is that of the haploid mosaics (Bridges, 1925a). In an earlier paper in the same year Bridges said: "In the table of sex types of *Drosophila* the haploid is entered with the index 1.25, the same as that of the 2N, 3N, and 4N females. Unfortunately, the haploid individual has *not* been discovered. But it is plain from the view just given that the expectation for a haploid *Drosophila melanogaster* is that it would be a female in sex character." A fly has now been found of which part is male, and the rest, including an eye and a fore leg, is female in type. The cells of the latter portion, as indicated by the ocelli, are half as large as those on the male side of the body. This fact strongly indicates that the female portion is haploid, thus fulfilling the prediction.

The *Matthiola* haploid is unique in that it contains a very potent little fragment in addition to its seven normal chromosomes. The fact that it so clearly resembles the tetrasomic form is evidence that the effect of the single fragment in the disomic haploid plant is very similar to that of the two fragments in the tetrasomic diploid plant. In both, the effect of the fragment or fragments is greater than that of the one fragment in the trisomic type, and extreme Small plants result. This case, therefore, closely parallels that of female haploidy in *Drosophila,* and con-

stitutes a further confirmation of the genic-balance theory
of chromosomal effect in development.

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A SYNCEPHALUS THORACOPAGUS MONSTER IN SWINE[1]

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Introduction

TERATOLOGICAL literature covering anomalies in the human race as well as in domestic animals is rather extensive. In the literature which deals with conjoining it is quite clear that similar tendencies prevail in the different species, although there is much detailed variation within these general tendencies (Hirst and Piersol, 1893; Wilder, 1904; Williams, 1909). It was deemed advisable to report this specimen inasmuch as it differs in so many respects from those of similar type that have been placed on record. Throughout the discussion will occur such terms as "fused," "bifurcated," etc., but it is not the purpose of the authors to convey in any manner their significance in the field of embryology as they have been used for lack of more suitable terms. This paper is primarily morphological in character.

Wymann ('58-'59) has reported the dissection of a syncephalus pig. Carey (1917) and Williams and Rauch (1917) have contributed similar discussions of syncephalus thoracopagus monsters in swine, and Crampton (1926) illustrates and briefly reviews the external characteristics of a double twin in pigs.

The anomaly discussed in this paper was farrowed by a yearling sow and weighed 660 grams. Carey's specimen weighed 500 grams. Carmichael and Rice (1917) found that the average birth weight of pigs farrowed by yearling sows was 2.44 pounds (1,107 grams). The monster was farrowed at term (112 days) with eight normal pigs, but was rather immature. The twins were males.

One member was somewhat smaller than the other. The spinal axes length of the two was 17.5 and 18.2 centimeters, respectively.

The pigs were joined front to front from the umbilical cord to the angle of the mandible (Figs. 1 and 2). The heads were fused at an angle and laterally each having two quite complete maxillae, but only one mandible. The union throughout was accurately symmetrical, exactly the same parts being fused.

The umbilical ring was imperfectly formed. A portion of the intestine, which normally lies within the umbilical cord during early foetal life, had withdrawn, but a post-foetal umbilical hernia was in evidence which was apparently the result of a secondary protrusion of the intestine through the patulous ring (Ahlfeld, '80–'82). Williams and Rauch found that "as nearly as we can tell from the specimen (which was opened in 1895) there are two umbilical cords. This, however, is not certain. The reception of two blood supplies from the mother is exceptional," Reeves (1915). In common with the specimen of Wymann, Carey and Crampton this monster had only one umbilical cord.

Two well-developed fore legs and two well-developed rear legs were present in each member. Two normal eyes and two ears were located in each skull. Two nostrils were present in each snout.

External Variations

In the literature covering monsters in animal life it is not uncommon to find references to reversed symmetry and mirror imaging. No specific instance of this phenomena will be cited in this paper but cases suggesting it which appear may be compared with similar ones in literature covering this subject (Fisher, 1866; Bateson, 1894; Newmann, 1916, 1917; and Morrill, 1919).

The dental arrangement in monsters of the dicephalus type display some variation. Reese (1914) found in a double-headed calf that "in the right mandible the left

Fig. 1

half has four incisor teeth, the right half has three. In
the left mandible the right half has four teeth, the left
half has three, though one tooth is missing from each
half.'' In 1917 the same author reported on a two-headed
lamb that ''except for a marked lateral twist in the right
skull and a slight twist in the left skull these two organs
seemed normal.'' Smallwood (1921) finds in a two-
headed calf ''the four incisors on each of the four man-
dibles are present, well developed and symmetrically
distributed.'' Pigs when farrowed have normally two
temporary tusks or ''needle teeth'' in each right and left
maxilla and mandible. Dentition in the mandible on this

<p style="text-align:center">F<small>IG</small>. 2</p>

specimen was normal. It was also normal in the maxilla
of one of the members. In the member with the cleft
palate there were two teeth in the left half and a cluster
of five in the right half of the maxilla. Of these tusks one
was normal in size and location while the other four were
somewhat smaller and located in twos immediately to
either side of the larger one. The cluster was somewhat
crowded (Fig. 7).

Wentworth (1912) has found considerable variation in
the number of mammae in swine. He also found that the
variation due to the suppression of a nipple in a pair
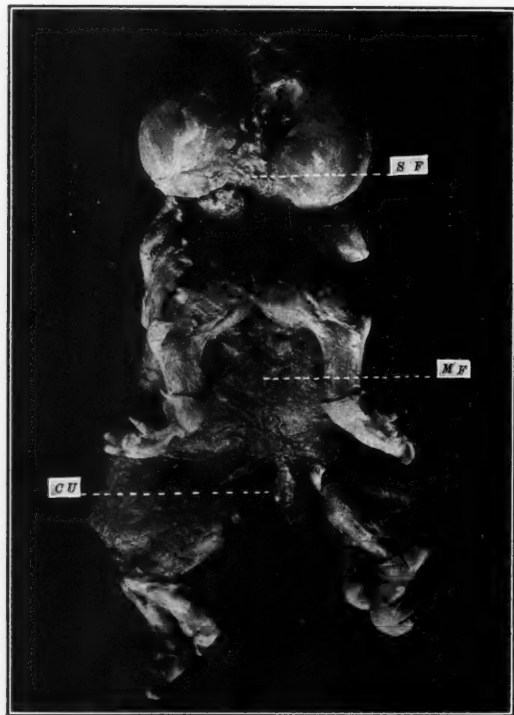occurred in the second pair from the front. One of the

Fig. 3

members of this pair had four complete pairs of mam-
mae with a spacing on the left side of 1.69, 1.64 and 0.98
centimeters, and on the right of 1.30, 1.92 and 1.20 centi-
meters. The other member had five pairs with one nipple
in the second pair suppressed, with a spacing on the left
side of 0.99, 1.12, 1.78 centimeters, and on the right of
1.30, 1.59 and 1.09 centimeters.

The figure showing the mandible (Fig. 2) will be re-
ferred to throughout the paper as the ventral aspect and
the position in Figure 1 will be known as the dorsal
aspect. The ears on the ventral aspect were normal in
size and location. The ears on the dorsal aspect were
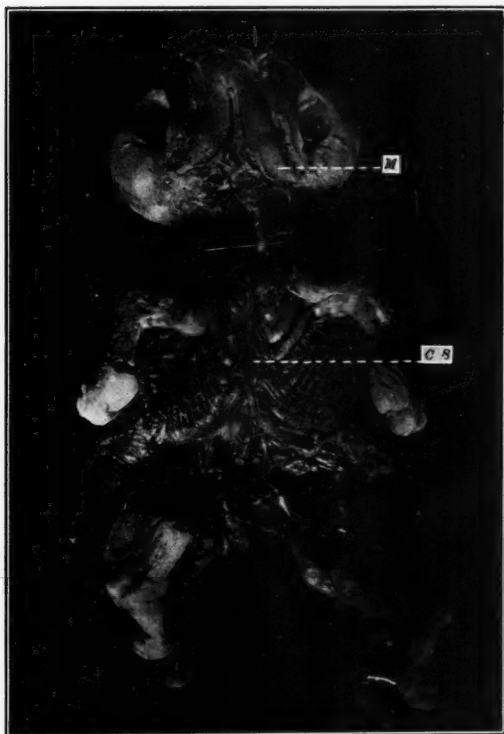conjoined at the external acoustic meatus. The promi-

FIG. 4

nence of the fused petrous temporal bones apparently interfered with the normal development of the funnel-shaped portion of the conchal cartilage so that the common external acoustic meatus was in plain view. Both of the ears on the dorsal aspect were somewhat smaller than were those on the ventral aspect.

Respiratory, Digestive and Genito-Urinary Systems

Wymann reported three nostrils on the snout, and lungs that were double. The specimen discussed in this paper had a normal respiratory system, except that in one of the pair the inner nasal processes (Fig. 7) of the
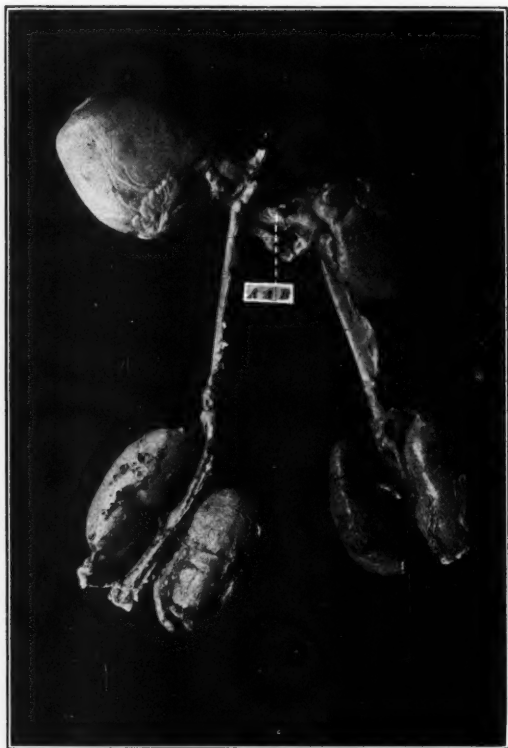
Fig. 5

intermaxilla had failed to fuse with the laterally placed
superior maxillary processes, resulting in a total cleft
palate. Each member had a well-developed trachea and
pair of lungs. In this connection Williams and Rauch
reported a single trachea connected to a ventrally lobed
lung mass, while Carey found a pair of lungs possessing
a corresponding trachea which continued cephalad into
its respective larynx.

Carey found a compound tongue. In this specimen
(Fig. 7) there was only one tongue. This was rather
thick and triangular in cross section, corresponding to the
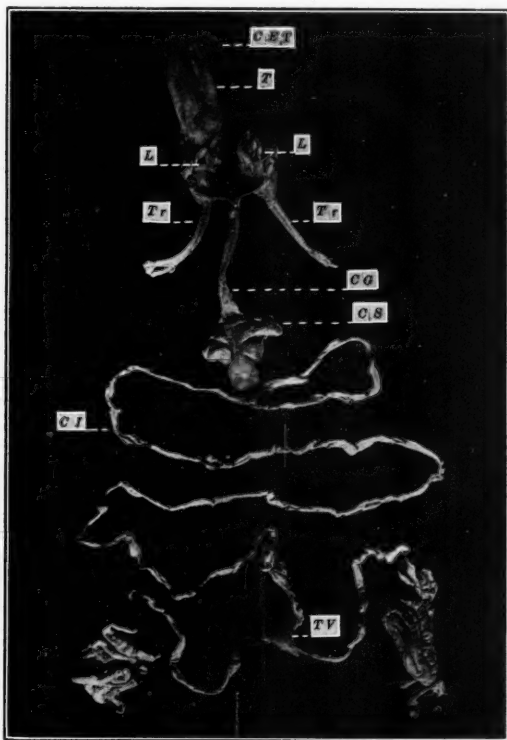peculiar shape of the oral cavity formed by two sets of

Fig. 6

maxillae and the single mandible. The point of the
tongue was curled off at an angle apparently for the rea-
son that it did not have sufficient room for full-length
development. It appeared to be at least one half centi-
meter too long.

The Williams and Rauch specimen had an alimentary
canal with a "divided pharynx, a grooved stomach, a
single pancreas and a single spleen." Wymann found "a
single alimentary tract to the lower one third of the ileum
and from there it was double." The single feature of the
alimentary tract of the Carey monster is quite compara-
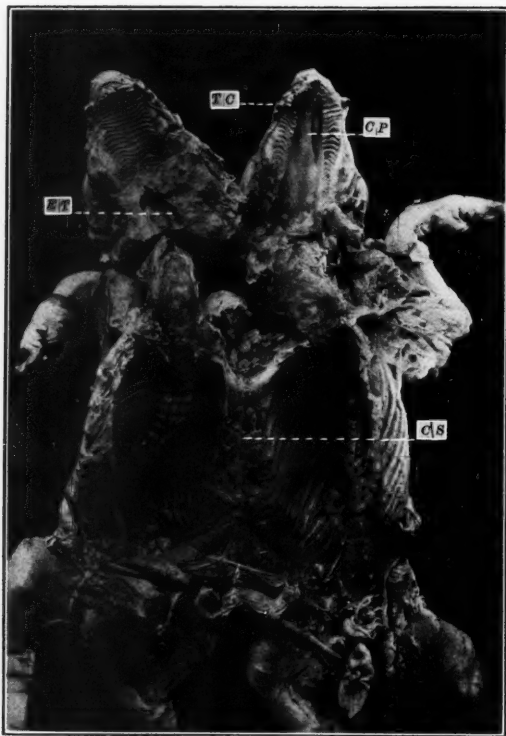ble with the alimentary tract in this specimen (Fig. 6)

Fig. 7

with the exception that he finds a bifurcation of the ileum a short distance cephalad to the cecum, each integer leading to its respective ileo-cecal valve. In place of a bifurcation in the ileum we found sixty-two centimeters from the stomach and twelve centimeters from the ileo-cecal valve a triangular vesicle varying from one to one and two tenths centimeters on each side (Fig. 8). The ileum did not proceed through this vesicle but connected with it at one of its angles. From each of the other angles the alimentary tract had developed normally for its respective body.

Complete duplicity of the pancreas and spleen prevailed. The livers, two in number, were attached to the
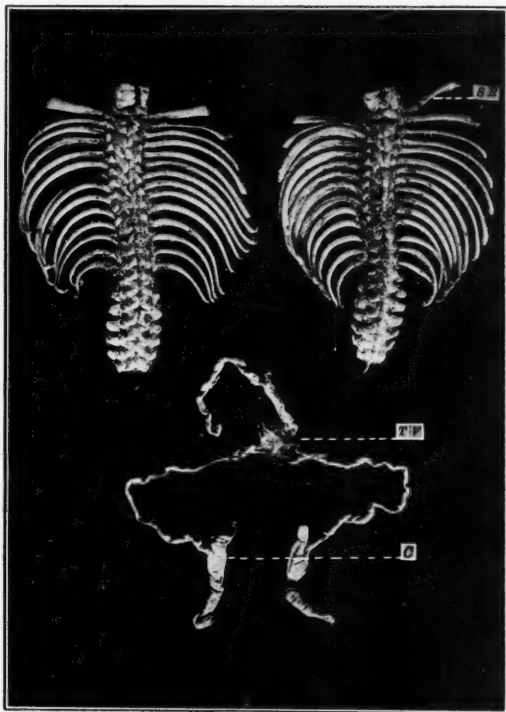
FIG. 8

common diaphragm and common stomach. There was one common peritoneum. Williams and Rauch found a single spleen and pancreas. Wymann reported that "the liver, kidneys, spleen and genitals were double." Carey found a normal pancreas for each body and two livers.

There was one large roomy pharynx and two complete larynges on the dorsal aspect. In the member with the normal palate one eustachian tube was abnormally large and led into and extended through the petrous temporal bone to the external ear. The other three eustachian tubes were normal.

The caudal end of the common gullet was much exaggerated in its dilation. There was also evidence of a par-

tial division inside of the cardiac end of the common stomach. This, however, may have been due to the heavy folding of the lining of the stomach. The stomach was flattened and triangular in shape, showing many deep folds and fissures in the interior wall. The pyloric end opened into the common intestine which emptied into the triangular vesicle described above.

The genito-urinary systems were double and complete. In one of the members one testicle was rather large and descended into the scrotum. The other three testicles in the pair were in the inguinal canal just below the abdominal ring.

Circulatory System

Wymann reported a "compound heart." Carey found "one large heart situated ventrad within the thorax in a separate pericardium. Dorsad and to the left was found a heart one quarter of the size of the ventral one. . . . The smaller one also possessed a separate pericardium." Williams and Rauch found "the heart a fused structure with two large thin-walled auricles and two large ventricles with equally thick walls." This specimen had two relatively flattened hearts, one somewhat smaller than the other, each in a separate pericardium. The aortic arches (Fig. 5), one proceeding normally from each heart, were connected with one large, and one small, anastomatic branch which provided direct communication between the two circulatory systems (Bishop, 1923).

Musculatory System

The posterior deep pectoral muscle of the left side of one member was fused symmetrically with the same muscle on the right side of the other member. This same condition prevailed on both the dorsal and ventral aspects. A similar fusion was observed in the aponeurosis of the obliquis abdominis externus, fascia of the obliquis abdominis internus, and the rectus abdominis muscles (Fig. 3).

Skeleton

Fusion of the bones in the skull was confined to the zygomatic processes of the squamous temporal bones

from the region of the malar, caudad through the petrous temporal bones. This was on the dorsal aspect only (Fig. 3). These bones were so rigidly fused that in an effort to separate them by applying force the surrounding structure yielded. Due to the fusion of these skull bones, the occipital bones on the fused side were dwarfed in their development, thus interfering with the normal alignment of the occipital condyles with the atlas.

The mandible, at first sight, appeared to be common (Fig. 4). The rami, however, were exaggerated in their divergence and close inspection revealed normal articulation at the respective condyles. The angle at which the halves of the mandible were attached to the respective maxillae would suggest that the two halves normally belong where they are located and that their complements on the dorsal aspect, or fused side, of the skull did not have room to develop. The fact that the anterior ends of these mandible integers came together rather normally may be explained on the basis of the crowded condition of the anterior end of the tongue as previously described. It will be observed in the above figure that the rami were somewhat strained or twisted. If the mandible is considered as common it is probable that we should associate each half as belonging to its respective maxilla.

One common thoracic cavity was surrounded by the combined rib structure of the pair. In both dorsal and ventral aspects the reflected right ribs met at common sterna, the reflected ribs of the left thus completing the skeletal encasement of the thoracic cavity. The twenty-eight ribs on each member were normally connected with fully developed thoracic vertebrae (Sisson, 1914). There were eight pairs attached to the sternum, a condition which is normal in foetal life (Hanson, 1919). Sisson finds when variations in the number of ribs occur (either fourteen or fifteen pairs) that this variation is ordinarily in the asternal pairs, the arrangement in post-foetal life being seven sternal pairs and seven or eight asternal pairs. In this specimen (Fig. 8) the sternal and asternal

rib pairs were normal. However, from the seventh cervi-
cal vertebrae of one of the pair there was developed a
short rib, which had characteristic sternal attachment.
The vertebrae were normal in number in both members
(Decker, 1915). There were two distinct cerebro-spinal
axes.

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M. Raeder for the accompanying photographs.

ABBREVIATIONS

A. A. B.—Anastomatic aortic branches
C.   —Caecum
C. E. T.—Curled end of tongue
C. I.   —Common intestine
C. G.   —Common gullet
C. P.   —Cleft palate
C. S.   —Common sternum
C. S.   —Common stomach
C. U.   —Common umbilicus
E. T.   —Eustachian tube
L.   —Larynx
M.   —Mandible
M. F.   —Muscle fusion
S. F.   —Skull fusion
S. R.   —Supernumerary rib
T. C.   —Tooth cluster
T.   —Tongue
Tr.   —Trachea
T. B.   —Triangular vesicle
U. H.   —Umbilical hernia
V. M.   —Variation in mammae.

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ALCOHOL AND SEX RATIOS IN MICE[1]

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PLAUSIBILITY is an argument that often makes a few data go a long way. This is the case with the experiments that have led to the claim that through the alcohol treatment of males, the sex ratio in a mammal can be controlled. The correlation between sex and chromosomes had been demonstrated; in mammals males were known to produce two types of sperm differing according to the presence or absence of a sex chromosome. Alcohol was believed to be an active agent, whether stimulating, depressing, or both. The only supposition needed was that the presence of a sex chromosome in half of the sperm brought about a differential reaction to alcohol. This provided a facile explanation of the experiments, for one might call upon a hastening or retarding effect on the sperm with the sex chromosome or upon the sperm lacking the sex chromosome.

But the plausibility of a result does not obviate the necessity of sufficient controls. However trite, it seems necessary to recall that balancing out the effects of all variables but the one under consideration is a primary requirement. Without an understanding of the action of normal variables it is never possible to be certain that all influential variables are controlled. When the causes of normal variation are understood vaguely, if at all, it becomes a matter of extreme difficulty to know how to plan an experiment. This applies to experiments upon the sex ratio in mammals, but the difficulty has not been clearly recognized. Under these circumstances the only

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[1] Presented at the Fifth International Genetics Congress, Berlin, September, 1927.

course is to equalize the test and control animals in every way possible, hoping in so doing to control the effective variables. The importance of this matter has led to the presentation of the methods here used, in considerable detail.

This paper presents data on the sexes of young mice from (1) experiments in which the fathers only were treated with heavy doses of alcohol fumes; (2) experiments in which the mothers only were treated with alcohol fumes, and (3) an experiment in which the fathers were treated with subcutaneous injections of alcohol. All the original data from the experiments under (1) and (2) are being published in *Roux's Archiv. f. Entwicklungsmechan. d. Organismen*; the experiment with fathers treated by the injection method (3) is reported here for the first time.

(1) Each of eight experiments with treated fathers consisted of four males from the same litter, two of which were confined in an atmosphere of alcohol fumes till completely anesthetized five days a week, starting at the age of four weeks, throughout the experiment. The other two males were handled and confined in the same manner except they never came in contact with alcohol fumes. These males were placed in a row of four mating pens, control and test alternating. Each one was mated with a group of four or five females three to four weeks older, from an unrelated strain. Females from the same litter were divided equally between test and control males. Litters of four females were preferred so that one could be mated with each of the four males. All females were examined daily for signs of pregnancy. Each pregnant mouse was given a box by herself; her ovaries were examined surgically to determine the number of corpora lutea and the morning the young were born she was mated with the males in the next box. As far as possible, each female was bred continuously until she gave two litters by each male. In this way the litters from each female were sired alternately by a treated and

a control male and half the mothers from the same litter
started with control males and half with treated males.
The young were killed and sexed on the day of birth.
After the males in five of the experiments had been
treated for thirty weeks a second set of females was dis-
tributed among them. In this set the females were mated
only once with each male. Four unit experiments were
undertaken with males from the Bagg albino line and
four with males from the Dilute brown line. In all cases
the females came from line 89.

(2) The treated and control females were litter mates
and both sets were mated with the same normal male (a
father or a brother, already shown to be mature at the
time the females were four weeks old and the treatment
started). During each pregnancy the corpora lutea were
counted, the young were sexed and killed on the day of
birth, and the mothers immediately remated with the
same male. Each female was bred as long as possible.
Mice from four different strains were used in this series
of experiments.

(3) In this experiment the males were treated with 0.2
cc. 20 per cent. alcohol injected under the dorsal skin on
alternate days (the same method of administration and
dosage as used by Bluhm and by Chaudhuri). First
pregnancies were used. The embryos were sexed four-
teen days after conception by binocular dissection. The
treated and control males were litter mates from line 89;
the females, F_1 hybrids between Bagg albino and Storrs-
Little, those from the same litter being divided between
test and control males.

Variations in prenatal mortality (based on corpora
lutea counts) have been shown to be independent of
variations in the sex ratio at birth by the analysis of both
the data from control litters as well as those from test lit-
ters (MacDowell and Lord, 1926). This eliminates the
possibility of an indirect influence of alcohol on sex ratio
through an increase in prenatal mortality, and warrants
the summarizing of all litters without regard to the
amount of loss incurred before birth.

TABLE I

INFLUENCE OF ALCOHOL TREATMENT OF ONE PARENT ON SEX RATIO OF THE OFFSPRING

Fathers		Mothers		Young					
Line	Treatment	Line	Treatment	Controls No.	Controls Per cent.	Tests No.	Tests Per cent.	Difference	Diff. P. E.
Bagg albino	inhalation	89 1st set	none	880	52.8	848	48.0	-4.8 ± 1.6	3.0
" "	"	" 2nd "	"	395	48.4	425	50.6	$+2.2 \pm 2.4$	0.9
Dilute brown	"	" 1st "	"	740	49.3	635	50.2	$+0.9 \pm 1.8$	0.5
" "	"	" 2nd "	"	261	52.1	245	50.6	-1.5 ± 3.0	0.5
			Total	2276	50.8	2153	49.5	-1.3 ± 1.0	1.3
Bagg albino	none	Bagg albino	inhalation	638	51.4	428	47.4	-4.0 ± 2.1	1.9
89	"	89	"	354	48.3	291	57.0	$+8.7 \pm 2.7$	3.2
C57	"	C57	"	393	52.4	264	54.2	$+1.8 \pm 2.7$	0.6
C58	"	C58	"	206	51.0	207	45.9	-5.1 ± 3.3	1.5
			Total	1591	50.9	1190	51.0	$+0.1 \pm 1.3$	0.1
89	injection	B. alb. × StoLi	none	547	52.5	621	48.0	-4.5 ± 2.0	2.2

The results given in Table I show that the differences are plus and minus; in five cases the actual percentage of males from a treated parent is lower and in four cases it is higher. In only two cases are the differences great enough to be considered statistically significant, namely, the treated Bagg albino males by the first set females give a significant reduction in the percentage of males, and the treated females from line 89 give a significant increase in the percentage of males.

These results must be considered in connection with the following results of previous authors working with mice: Bluhm (1924), Danforth (1926) and Chaudhuri (1927) report an increase in the proportion of males from treated fathers; Gyllenswärd (1923) obtained a lower percentage of males from treated fathers; Crew (1925), with larger numbers, obtained no modification of the sex ratio in ten generations of treated fathers; and Bluhm (1926), in repeating her experiment with larger numbers and with the variables more fully controlled, did not obtain a result that was statistically significant according to her own calculations.

Bluhm has suggested that the differences between strains may account for the divergent results. As soon as strain differences are accepted, the simple physiological hypothesis that the presence of the X-chromosome (or any other feature distinguishing male and female determining sperm) causes a differential reaction of sperm to alcohol, falls to the ground. If alcohol affects sperm in different lines differently, it is the genes carried and not the presence of the X-chromosome as such that accounts for the reaction to the alcohol. This leads to a genetic hypothesis that some strains carry genes that determine alcohol resistance in sperm, other strains carry genes that determine alcohol susceptibility in sperm; some strains have neither kind of genes; these genes may be sex-linked, and express themselves in sperm carrying them. If we consider that alcohol depresses the motility of sperm, in strains carrying the alcohol-resisters in the

sex chromosome it will depress the male-determining
more than the female-determining sperm; in strains car-
rying the alcohol-susceptible genes alcohol will depress
the female more than the male-determining sperm; and
in strains carrying neither type of gene, or at least not
carrying them in the sex chromosome, alcohol will have
no differential effect. This must be further complicated
by assumptions to explain why the same albino males,
after giving a reduction in the percentage of males with
one set of females, when mated to a second set of females
closely related to the first, showed no modification; and
still further assumptions are required to account for a
modification of sex ratio by treatment of females. With-
out formulating these special assumptions, it is evident
that this whole hypothesis is both complicated and highly
speculative; it would be difficult to test. Moreover the
assumption that genes are active in sperm is questioned
by the evidence of Muller (1927). The bountiful evidence
of the influence of genes on the gametes carrying them in
plants can not be used for animals, for, as Mohr (1925)
has pointed out, the history of the gametes in plants and
animals is not parallel. Whereas in plants the pollen cell
grows, the pollen tube is developed, and divisions of the
haploid chromosome take place, in animals the chromo-
somes are carried by the spermatozoa in the resting con-
dition, from the sperm mother cell to the ovum; rather
than growth, the transformation into the spermatozoan
usually involves actual loss of cytoplasm.

Taking all these results together, there is no question
but that they conflict; from treated males, more males and
fewer males; from treated females, more males and fewer
males; and the cases showing no effect at all are numer-
ous. This looks very much like a random distribution.
In all cases the modifications observed are small and those
taken to be significant are only just over the low limit of
statistical significance. The amount of normal variabil-
ity actually shown between different litters is not mea-
sured or taken into account in any way. These considera-

tions of the results themselves question the acceptance of the statistical significance as biological significance. If the biological significance of the results is doubted, the complicated assumptions suggested and the difficulties involved in the interpretation are eliminated, for all the results will agree with the hypothesis that the differences in sex ratio observed lie within the limits of the normal variation. Test and control ratios in a given experiment differ when the causes of the normal variability are not fully controlled by the methods of the experiment. Until the causes of the normal variability in the proportion of the sexes are known, it will be a matter of chance whether they are eliminated from a given result.

We conclude (1) that as alcohol has no general effect on the sex ratio in mice, no practical application is promised; (2) that if alcohol will give various results according to genetic constitution, the genetic control of the sex ratio is primary and the significance of any influence of alcohol is lost; (3) a wider knowledge of normal variables is needed before alcohol or any other experimental agent can be demonstrated to cause small modifications in the sex ratio.

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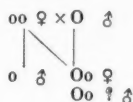   1927. *Zeitschr. f. ind. Abst. u. Vererbgsl.* 43.

GENETIC EVIDENCE FOR DIPLOID MALES IN HABROBRACON[1]

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THE first work on *Habrobracon juglandis* (Ashmead) was done with wild black-eyed stock. From this material it was observed that virgin females produce only males while mated females produce both males and females. The conclusion was drawn that all males come from unfertilized eggs.

With the discovery of a recessive mutation, orange eye color, this could be more adequately checked and results proved the above conclusion to be incorrect. From the cross of recessive orange females by related type males there appeared orange azygous males, black heterozygous females, and a few black anomalous males which must have received their eye color from the male parent.

$$oo \; ♀ \times O \; ♂$$

$$o \; ♂ \quad Oo \; ♀$$
$$Oo \; ? \; ♂$$

The black males, with the exceptions mentioned below, when crossed to orange females, produced but few daughters. These had black eyes like their fathers and were almost entirely sterile.

Since it was believed that males in Hymenoptera must be haploid, it was postulated that either the sperm nucleus alone had undergone cleavage in the egg cytoplasm and formed the entire embryo or that sperm and egg nuclei had developed independently, resulting in the formation of a haploid mosaic. The theory of mosaicism was strengthened by the appearance at this time of six black anomalous males that bred as orange and one
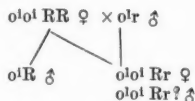
orange-eyed brother that bred as black. These were fully fertile. No other such individuals have occurred since. The facts that the majority of patroclinous males were comparatively sterile and their few daughters almost entirely so remained unexplained.

A study of several new recessive mutations has thrown additional light upon the subject. A series of quadruple allelomorphs affecting eye color, black (O), light ocelli (o^l) (conveniently known as "light"), orange (o), and ivory (o^i), and three pairs of simple allelomorphs affecting wing character, defective venation (d), wrinkled wing (w) and reduced wing (r), have been found. These four loci segregate independently.

In any type of cross between related stocks where the female is homozygous for one or more dominant and one or more recessive factors and the male carries the allelomorphs there are produced some anomalous males. Two types of such matings will be discussed in detail.
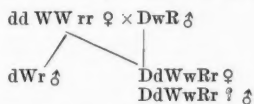
If a female, homozygous for a recessive eye color and a dominant wing character, is crossed to a male carrying allelomorphs there are produced three classes of offspring, haploid matroclinous males, diploid biparental females, and a few males resembling their sisters in possessing both dominant characters. For example, an ivory-eyed normal winged female (o^{i}o^{i}RR) when crossed to a male with light ocelli and reduced wings, (o^{l}r) gives ivory sons, (o^{i}R), daughters with light ocelli, (o^{l}o^{i}Rr), and a few sons with light ocelli, (o^{l}o^{i}Rr ?) all with normal wings.

$$o^lo^l \text{ RR } ♀ \times o^l r ♂$$

o^{i}R ♂   o^{l}o^l Rr ♀
              o^{l}o^l Rr? ♂

These light males have, without any question, received a head character from their father and a thorax character from their mother and so can not have been derived from the sperm nucleus alone but are biparental. If they are mosaics it is difficult to explain why we get no combination of the recessive characters which would give ivory

reduced males. An additional point of interest is brought out by this cross. Light females, heterozygous for orange or ivory, have ocelli with less pigment than do males and females of light stock. The biparental males in the above cross likewise have lighter ocelli than ordinary haploid light males. This fact suggests that they are diploid like their sisters.

Stronger evidence for diploidy of biparental males appears when three character differences, all affecting one structure, the wing, are involved. If two recessives and one dominant are contributed by one parent and the allelomorphs by the other, the wings of the biparental males always show all three dominant traits. For example, homozygous defective, reduced females (ddWWrr) when crossed to wrinkled males (DwR) give defective reduced matroclinous sons, (dWr), type daughters, (DdWwRr), and a few type sons (DdWwRr ?).

dd WW rr ♀ × DwR ♂

dWr ♂   DdWwRr ♀
                 DdWwRr ? ♂

It is difficult to conceive of any relationship of chromosomes other than diploidy which will explain this.

About two hundred biparental males have been tested, many of them several times, by mating to recessive females. Over seventy-five per cent. were entirely sterile and the remainder have produced but few daughters. With the exception of the mosaics mentioned above and of one male which bred as a heterozygote they have all produced only dominant daughters and so have bred like azygous or homozygous males.

There is a high percentage of morphological abnormalities among the dominant daughters of biparental males, and although most of them lay numerous eggs only a few hatch. From considerably over one hundred tested there have resulted one completely sterile male, several lethal larvæ and pupæ, and one daughter that proved fully fertile. As far as could be recognized these offspring also showed only dominant characters.

The exceptional biparental male which bred as a heterozygote serves as an additional bit of evidence for male diploidy. He produced three daughters, each different, showing that he had at least three types of spermatozoa. One contained a recombination of his parental factors.

A careful study of the external and internal morphology of the biparental males discloses no evidence of intersexuality, no tendency towards femaleness. They are normal males in both appearance and instincts.

The first question that presents itself is, "Why are these biparental, apparently diploid individuals, males?" A logical answer at present is postulation of an X chromosome as in many other forms. Further research may disclose a mutation behaving differently from those already studied and indicating a sex chromosome which, by its haploid or diploid condition, determines sex more or less irrespective of other chromosomes involved.

Cytological work upon Habrobracon is difficult. Haploid males possess about eleven very minute chromosomes, diploid females about twenty-two. Spermatogenesis is like that of the hornet. The first division is abortive, the second apparently produces two functional spermatozoa. Abortive first maturation divisions have also been observed in the testes of biparental males and it is conceivable that this process would result in diploid sperm, an idea thoroughly consistent with the genetic results. Daughters of biparental males would then be triploid, which might account for their high percentage of abnormality and sterility.

The question of the method of sex determination in Hymenoptera, which seemed to be settled so long ago, bids fair to be one of the last to be fully understood.

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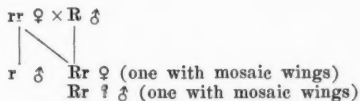
THE RELATION BETWEEN GYNANDRO-
MORPHISM AND MUTATION IN
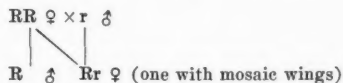# HABROBRACON[1]

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In *Habrobracon juglandis* (Ashmead) gynandro-
morphism and other types of mosaicism occur as rare
phenomena. The previous speaker has dealt with the
production from fertilized eggs of males which are bi-
parental and presumably diploid. Although numerous
such exceptional biparental males regularly appear there
have been found only five exceptional uniparental
females developed from unfertilized eggs. The produc-
tion of these females may be explained by postulating
suppression of the second oöcyte division with resulting
diploid parthenogenesis. (n tetrads in egg divide into n
dyads in first polar body and n dyads in egg. The latter
separate into 2n monads, each of which divides in the
first cleavage.)

Three biparental diploid mosaics—one female and one
male produced by females with reduced wings crossed to
type males

rr ♀ × R ♂

r ♂   Rr ♀ (one with mosaic wings)
       Rr ? ♂ (one with mosaic wings)

and one female from the reciprocal cross

RR ♀ × r ♂

R ♂   Rr ♀ (one with mosaic wings)

[1] Presented at the Fifth International Genetics Congress, Berlin, Sep-
tember, 1927.

resembled their type sisters except that in each case one wing was reduced. The females were fertile, while the male, like other diploid biparental males, was sterile. These three cases may be explained by postulating somatic mitotic irregularity.

Fourteen uniparental (?) haploid mosaic males have been produced by heterozygous mothers. In six of these cases the mothers were virgin and in three others they were producing no daughters, so that the uniparental nature of the majority at least is certain. Three of these males were mosaic for wings (one long, one reduced), while nine were mosaic for eye color. The remaining two were not obviously mosaic but proved so by breeding tests. Of the eleven producing offspring each of seven which were mosaic for one factor produced but one type of progeny: one mosaic for three factors produced but one type: and each of three produced two types. Especial interest attaches to these three cases in which a male produced more than one type of sperm. One male, mosaic for one factorial difference, produced daughters of the two types in numbers 25 and 374, respectively; another, mosaic for two differences, produced only two types, one and 284, respectively; while the third mosaic for four differences likewise produced only two types, 11 and 309, respectively. Unlike the heterozygous male mentioned by the previous speaker, these mosaics produced but two types of sperm representing but two combinations of the maternal complex.

The presence of two types of tissue, genetically different, in the bodies and gonads of these males may be explained by egg binuclearity. The inclusion of two oöcytes into one egg has been suggested to explain mosaicism in certain Lepidoptera. Failure of extrusion of the second polar body is a reasonable alternative. In this case the two oötids resulting from the second maturation division take part in parthenogenetic cleavage. Post-reduction of genetic factors in oögenesis must be assumed. (Tetrad OOoo divides into dyads Oo in first polar body and Oo in

egg.   In the second oöcyte division nuclei are formed containing O and o respectively.)   That pre-reduction of other factors also takes place is shown by the fact that nuclei differing in certain genes are yet similar in others for which the mother was heterozygous. (Tetrads OOo'o' and RRrr divide into dyads Oo' and rr in the first polar body and Oo' and RR in the egg.   In the second oöcyte division nuclei are formed containing OR and o'R respectively.)   More convincing evidence for simultaneous post- and pre-reduction was shown by one of the impaternate females.   A female heterozygous for three genes produced an impaternate daughter homozygous for two and heterozygous for the third.   (Tetrads OOoo, DDdd and WWww divide into dyads OO, dd and Ww in first polar body and oo, DD and Ww in egg.   Sons of this impaternate female were orange non-defective non-wrinkled and orange non-defective wrinkled in approximately equal numbers.)

Nineteen gynandromorphs have been found.   Fourteen had male heads and female abdomens (with invasion of male parts into anterior portions of abdomens of three); three had mixed heads and female abdomens; one had female head and mixed abdomen, and one had female head and male abdomen.   Eleven had homozygous mothers and eight had heterozygous (two for one gene, six for two genes).   Unlike the mosaic males all were produced by mated females.

As regards origin of male and female parts of body, eight were of no significance as similar factors occurred in both parents.   One had female parts showing the dominant paternal trait and thus presumably of bisexual origin.   Male parts were matroclinous in nine including six in which the mother was recessive or heterozygous.   Since the male parts showed the recessive trait they were in these six cases certainly of unisexual origin.   One with male parts patroclinous is regarded as a diploid gynandromorph in which male parts are comparable with the diploid males discussed by the previous speaker.

Gynandromorphism may be explained by egg binuclearity with fertilization of one nucleus. The diploid gynandromorph, however, may have resulted from fertilization of both nuclei.

Production of orange eye color has occurred in ten cases in which only black and ivory were possessed by the parents. Ten males from heterozygous mother, Oo^i, and three gynandromorphs from ivory females, o^io^i, by males breeding as black, O, are of interest in this connection. One of these ten males with ivory eyes produced both black and ivory daughters and two of the ivory gynandromorphs had black fathers. These facts show that these three ivory-eyed individuals were mosaic for black and that ivory tissue is not necessarily changed to orange by association with black tissue in the mosaic. In the remaining seven cases, however, orange color appeared. Four males and one gynandromorph had eyes mosaic for black and orange (two of the males bred as black, one as ivory, and one as black and ivory). It may be supposed that here proximity of black facets caused the ivory to take on an orange appearance although in one of the males the compound eyes were pure orange, black appearing in the ocelli only. Five males had eyes entirely orange. Two of these bred as black and were therefore mosaic by test. The remaining three were certainly mutants producing orange daughters only and showing no evidence of mosaicism. Whether these arose from mosaic embryonic tissue and were comparable with the others, and whether the orange in the mosaics is a true mutation which happened not to involve germ tissue are questions as yet undecided.

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INJURIES TO VEGETATION BY MOUND–BUILDING ANTS

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MANY sorts of ants have intricate interactions with vegetation that may be benefited or injured thereby and our common "carpenter ant" is well known to injure timbers as well as standing trees by its excavations.

The mound-building ants of the eastern United States frequent wooded areas and gain much of their food from searching the trees for insects and honeydew made by plant-lice, scaly bugs and tree hoppers. What effects these ants have upon vegetation is not at all self evident: as they kill many small green worms and other enemies of leaves they might be thought beneficial to the vegetation; but as they defend the insects that suck the juices from the branches they abet these injurious insects and to some extent aid in the detriment of plants infested by plant-lice and other suckers of juice; however, as Wheeler remarks, the honeydew thrown off by the plant-lice would tend to injure the leaves if not removed as by ants so that the ant is not convicted of great injury after all.

Any direct attack upon plants by these mound-building ants, *Formica exsectoides,* seems not to have been suspected till in 1912 when Clinton[1] first noted the dying of trees about the mounds of these ants.

The affected trees were but small ones and showed near the ground not only moulded areas but constrictions of the trunk.

These ants are not vegetarians and any destruction they may cause is not from eating leaves or wood, nor do

[1] G. P. Clinton, "Notes on Plant Diseases of Connecticut," 36 Report Conn. Ag. Exp. Sta., p. 354, pl. 19a.

they dig into trees and channel them out, hence one would not suppose that it was the ants had killed the trees.

As moulds were found on the lower parts of these small dead trees there was probability that the mould was the real cause of the destruction.

In 1914, Graves[2] got nine species of fungi, chiefly Fusicoccum from the bark of the infected trees and thought death due to a new kind of fungus of the white pine tree, but did not succeed in inoculations with these reputed destructive agents. G. P. Clinton ascribed the same trouble to other fungi a species of Phoma, resembling the *Phoma abietina,* causing the disease of spruce called "Einschnürungskrankheit" from the pinched-in appearance of the trunk of the tree.

W. O. Filly, the state forester of Connecticut, stated in 1915 that in the State Forests at Portland the white pine plantations showed rounded blank areas and almost always there were these ant hills at the center of each dead area.

In 1916 Hawley and Record[3] came to the conclusion that in reality the ants were the cause of these dead regions in the plantations. They found young trees dead near the mounds of *Formica exsectoides* in about sixty cases in New Haven, Ansonia, Middlebury and Union in Connecticut, in Pike County, Pa., as well as in Massachusetts, New Hampshire and New York states.

The trees were chiefly white pines, two to fifteen years of age, but there were also Scotch pine, red cedar, aspen, shag-bark hickory, gray birch, bear oak and staghorn sumac killed in these circular areas that measured on an average ten, but up to twenty feet in radius. Up to forty trees might be killed about one mound. In one pine plantation with very many ant mounds seventy-five per cent. of the trees were dead.

Photographs showed that the trees were killed near the ground and swollen about the injured place; but there

[2] A. H. Graves, *Mycologia,* Vol. VI, No. 2, March, 1914.

[3] R. C. Hawley, and S. J. Record, "Do Ants Kill Trees about their Colonies?" *American Forestry,* Nov., 1916, Vol. 22, No. 275, pp. 685–6.

was no sign of biting or of eating of the bark. The
cambium of the trees seemed girdled as by a canker.

Hawley and Record concluded that the only way to
stamp out the trouble was to destroy the ants, as by car-
bonbisulphide or by naphthalene flakes, but if the ants
were not exterminated young trees should not be planted
within twenty feet of each mound of *Formica exsec-
toides* F.

Meantime, in 1915, Haasis[4] studied the problem and
came to a different conclusion.

He observed ten ant hills in white pine stands of some
seven years' growth and six of these were in dead blank
spaces and four in the midst of living trees. His illustra-
tions show the elliptical reddish sunken areas on the bark
of the trees about the lenticels, and toward the ground
some indications of gnawing. *Formica exsectoides* was
seen gnawing the bark of a diseased tree and the author
states that Filly had seen ants feeding at the base of a
dead tree. Moreover Haasis found ants quite common
tending aphids on trees of various sizes. He found the
destruction centered about the ant mounds: there being
concentric circles, the dead trees near to the mound, then
trees with colored leaves and back of these the normal
green trees.

His summary and conclusions are about as follows:

    (1) The disease is associated with ant hills.
    (2) There are generally infestations of fungus and
        of Scolytid beetles.
    (3) The destruction may be found about the hills
        both of *Formica exsectoides* and the black ant
        Formica fusca var. *subsericea* Say.
    (4) Both sorts of hills may occur with no damage to
        trees.
    (5) The death of trees occurs both in plantations
        and in the wild state.

[4] Ferdinand W. Haasis, ''Dying of Young Pines in Circles about Ant-
hills,'' *Journal of Forestry*, XV, Oct., 1917, No. 6, p. 763–771.

The trouble is probably due to fungus, but ants are probably connected with the spread of the disease. The gnawing they do seems to be a minor factor.

It remained for H. B. Pierson,[5] State Forest Entomologist, Augusta, Maine, to bring conclusive evidence of the guilt of the ants in these destructions of young trees.

Pierson states that in New England no live trees or shrubs are found close to these ant mounds that stand almost always in open glades in the woods; moreover these open areas about the mounds grow larger as the mounds grow larger. In 1919 he was able to see the damage done to the pine trees in the Harvard Forest in Petersham, Mass., the stems from three up to seven inches above the ground were sunken in about one quarter of an inch, and swollen above and below these lesions that centered about lenticels.

In 1920 he mapped and measured the areas of injury and found the mounds generally in groups on terraces of hillsides but not on north exposures, and always on sandy loam, except a group of 25 on a talus of sandstone.

These groups of mounds often contained from 8 to 30 mounds each some three feet in height by six in diameter.

Pierson thought the damage done greatly exceeded the usefulness of these ants, though he says that they capture and drag in beetles, flies, moths, grubs and caterpillars in addition to their chief food the honeydew from aphids or plant-lice, colonies of which were almost always found being tended by these ants on trees close to the mounds. White pine trees over six feet were rarely killed.

The sorts of trees killed were, *Pinus strobus, Pinus sylvestris, Juniperus communis, Juniperus virginiana, Populus tremuloides, Hicoria ovata, Betula populifolia, Quercus licifolia, Pyrus malus* and *Rhus hirta.* In most localities even the grass and low bush blue berry and

[5] H. B. Pierson, ''Mound Building Ants in Forest Plantations,'' *Journal of Forestry,* Vol. XX, No. 4, April, 1922, p. 325–336.

Idem, ''Insects Attacking Forest and Shade Trees,'' *Bulletin* No. 4, Maine Forest Series, 1923, Augusta, Maine, p. 37–38, 2 figs.

other small plants and shrubs, were killed for a distance of several feet around the mounds. Besides the annular lesions on the pine trees, lesions on one side of the poplar trees weakened them and led to their being broken off by the wind. In the apple and the sumac the injury was by defoliation, the ants chewing the petioles.

In a small scrub apple the ants showed decided sense of direction; one branch especially casting a shadow on the mound was almost the only one attacked and in a week the ants had most all the leaves off. Though the ants did some damage on other branches they seemed to think it hopeless to remove the leaves from the whole tree.

This supposed element of shade and sunshine is again evident in the author's statements that the maps made of the dead areas in the young forest show that the ants kill trees to get the sunlight; the greatest damage being east, west and south of the mounds. This desire for a place in the sun is emphasized by the author who says: "As soon as a shadow of a tree was cast on the nest for any length of time, that tree was attacked." Though Pierson found a species of Phoma at the lesions near the base of the dead tree, he surmised that the ants might produce these lesions with formic acid; and he, making a watery solution from many ants, injected this into living healthy trees near the base and within a week obtained lesions identical with those in dead trees about the mounds.

Continuing these experiments at Amherst State College with potted white and red pines in a greenhouse during the winter and using commercial formic acid Sp. G. 1.06 that seemed weaker than the solution from the ants, he injected this into the stem, or else applied it on cotton after making many needle holes in the cambium, or even without punctures (covering cotton with paraffine to prevent evaporation). Microtome sections of the stem after one to three weeks of such treatment showed ducts abnormally swollen, even bursting as a typical emphysema while the long cells of primary cortex and true phloem had collapsed.

The cells had been killed down to the xylem or wood. Apparently formic acid had coagulated the protoplasm as it does the white of egg. However, not until the following spring did Pierson see the ants actually attacking the pine trees and then only after days of waiting. "Climbing the main stem the ants begin to chew and tear away the epidermis and the cork cambium surrounding the lenticels, meanwhile squirting acid into the wound." This formic acid penetrates and coagulates the living cambium and stops the downward flow of sap thus giving rise to the swelling above the lesion of attack. The injury is superficially like a fungus canker.

Having thus come to the conclusion that these ants are inimical to the forester, Pierson states that it is useless to plant pines within fifteen feet of a mound and that the ants may be eradicated if a pound and a half of carbon bisulphide be put on top of the mound with holes made to let the heavy gas penetrate and kill the ants. He also suggests that satisfactory results in destroying these ants might be got by infection with bacterial diseases, since the ants feed one another and hence might spread such disease as is caused by *Bacillus acridiorum,* which Marshall[6] states annihilated a small species of ant near Paris in 1911; as was the case also for other ants in South America.

In Baltimore County, Maryland, facts confirming the conclusions of Pierson, that these ants do kill plants, were obtained by the present writer from 1905 to the present time from repeated observations of many mounds.[7] For twenty years bare areas of sterile earth left from mining operations were seen in process of natural invasion by weeds and trees accompanied by increase and migration of ant mounds.

Amongst nearly two hundred mounds examined some had grass and weeds growing upon them but many were

[6] Marshall, C. E., "Microbiology," Amherst, Mass., 1912, pp. 637–643.

[7] E. A. Andrews, "Sequential Distribution of Formica exsectoides Forel," *Psyche,* XXXIII, 1926; "Ant Mounds as to Temperature and Sunshine," *Journ. Morphology,* v. 44, 1927.

kept clear of vegetation, year after year, and some showed a cleared area about the base extending out over the level, six to twelve inches from the mound itself. Where Japanese honeysuckle is abundant and covers up all it encounters its failure to overgrow prosperous mounds is the more striking since when any mound is abandoned by the ants it will be soon concealed by overgrowing honeysuckle.

Ants were seen to nibble the leaf stems and the growing tips of the honeysuckle, so that they died back from over the mound and the cleared zone. In starting a new mound by colonization these ants were seen to mine into the earth amidst small weeds and later to suddenly kill off all the plants within the area of excavations and mound foundations. There was a strong tendency to kill off all that could be killed upon or close to the mound as it progressed year by year, so that in general the prosperity of the community was indicated by the absence of vegetation over the mound and its external zone about the base.

Grass, however, often becomes permanently established upon mounds in the sun, but on occasions, even that may be after some years cut down and pieces even as long as 4½ inches were cut off and dragged up to be added to the materials of the mound. In one case a fine mound nestled against the south face of a dense mass of catbriar that tended to grow out in the air some feet above the mound and here was presented the remarkable sight of ants at work nibbling the very tough stems of the leaves that were above the surface of the mound and far from it, as if the ants were aware that the leaves came between the mound and the sky.

Observations made after a forest fire in 1925–6 had destroyed much vegetation but left stumps that sprouted as the area was populated anew showed undoubted death of trees both seedlings and sprouts by attacks near the base, as described by Pierson, in addition to the nibbling of leaves of grass, leaves of blackberry, catbriar and honeysuckle.

A list of the trees and non-woody plants thus girdled or otherwise attacked by these ants and ultimately dying is as follows:

Black Locust	Dogbane milk-weed
Maple	Canada thistle
Tulip	Tall whitish clover stalks
Aspen	Plantain flower stalks (''Rib Grass'')
Wild Cherry	Yarrow
Dogwood	Goldenrod
Sassafras	Summer aster
Catbriar	Ironweed
Grass	Asparagus
Daisy	White Melilot
Blackberry	Sundrops
Wild carrot	

That these ants actually attacked plants near the bases was observed in some cases though the observed attacks were infrequent and of intermittent nature.

In some cases ants were seen to nibble small black locust trees near the base at the same time that fellow ants were busy upon the tree in collecting honeydew from the tree hoppers, Vanduzea, and subsequently these trees died leaving the bugs to suffer from lack of sap and thus depriving the ants of the use of the honeydew from these small trees. In one case the base of a large tree very much frequented by ants for the same source of honeydew was occasionally nibbled now and then by ants that stopped in passing, though here the trunk of the tree was six inches in diameter and 25 feet in height. In attack upon bark of this tree one ant bent down in front so that jaws widely opened were drawn from side to side like a rasp for ten minutes without cease, the posture showing great muscular work. The same ant then turned to a nearby spot and rasped for three minutes, then to another very near spot for a few minutes and then suddenly hastened from the tree altogether.

Three trees four to twelve inches in diameter standing close together just south of a fine mound (No. 6) were not used by the ants since they were two dogwood and one nut tree that gave no honeydew and were traveled

over by but few ants: nevertheless on some occasions, of
the many ants that passed by on excursions out and back
to the mound, some lingered at the bases of these trees
and on different occasions were seen to stand nibbling at
the old dry bark for periods of 10 to 20 minutes, steadily
and most energetically. Fifty attacked July 15, none
July 24, but again many August 8. From time to time
these ants turned forward the part of the body capable of
spraying formic acid and actually did spray the region
cut by their jaws as was evidenced by applying to that
region paper made blue with litmus solution, which then
turned red over places ants had nibbled, but not else-
where nor where bark was cut by a knife. Litmus paper
also turned red where ants were seen to attack small
trees.

These three trees standing close to the mound cast a
dense shade upon it and it might be supposed that the
attack of the ants had reference to this fact for there are
good grounds for concluding that this kind of ant though
it builds amongst trees succeeds best when not in dense
shade and in general occupies natural or artificial glades
or open spaces and that when the trees grow large enough
to shade any mound overmuch, the ants either migrate to
a new mound or else fail to maintain a prosperous
existence.

As above stated, Pierson sees a direct connection be-
tween the shading from sunshine and the killing of young
trees: however, in Baltimore County no such close de-
pendence was observed. The trees killed were at all
points of the compass with reference to the mounds and
had they grown some would and some would not have
shaded the mound; most of them did not at all interrupt
the sunshine at the time of attack.

The annual plants that were killed often stood so far
from the mounds that they could not shade them this year
and they would not have done more another year.

The extreme radius distance for this ant destruction
was 35 feet. Also the attacked trees and plants were

mingled with others that escaped attack. All plants attacked were scattered about mounds, not in any one direction.

The attacks are long continued and the results often apparent only after much time has elapsed from the first injury so that some maples showing premature coloring of the leaves in September were found to have been injured near the base by ants at some early period of the summer.

That such attacks need not be directly correlated with shading was also seen in experiments in which sticks and wires wrapped in filter paper were planted in mounds and these were nibbled and irrigated by the ants from time to time through long periods, though they cast little or no shadow, in particular a stout iron wire about two feet high wrapped in filter paper, blue with litmus and tied with black thread, was thrust into a large mound and left two months. Any such object is immediately overrun with ants that bite and irrigate it, if there is any motion especially, but after the novelty is worn off but few ants visit it. In this case, variable numbers, but few, ants were found on this rod during many days nibbling at the threads till some were cut into floss and also biting the free edges of filter paper. In two months much of the *surface* of the filter paper above had been roughened by the jaw-work of the ants and many holes eaten through the paper. As the jaws are but dull, long periods of work and much effort were needed to accomplish this work.

While motion of an attacked object is potent stimulus, inactive objects such as vaseline, milk, blood, white or yolk of hen egg are likewise set upon, bitten and irrigated.

The assumption that these ants here in Maryland destroy plants merely on account of the shade they cast lacks sufficient evidence to support it. Attack is a mere general phenomenon. However, the general habit of attacking vegetation round about the mound would often lead to deferring the time when the trees would grow up enough to shadow and injure the ant community and hence in the

long run this tendency to destroy small trees would be of benefit to the race, even though much waste of labor were involved.   Though eventually the community might be killed out by shade, the period of enjoyment of sunshine might be prolonged by the delay in reforestation caused by such ant attacks in the neighborhood of mounds.

The following characteristics of the attacks of these mound-building ants upon living plants invite speculations:

The attacks bring no immediate good, neither food nor water, nor material for building, and no use is evident unless in the long run some of the attacks may lead to less shading of the mound.

Attacks may even be harmful as when food-bearing trees are killed.

The attacks are individual work of some ants, not the concerted work of all present as in mound-building or in food-getting.

The attack is interrupted and irregular in time and in space; some trees escaping, others, apparently at random, being attacked and the attack is on some days and not on others.

The attack involves great muscular strain and employment not only of the jaw muscles and acid glands but muscular activities of the legs, neck and thorax.

The attack is persisted in for many minutes and then suddenly abandoned and not carried through as in food-getting by killing-insects.

The attacks center about the mound and are more intense on objects upon the mound and less on those away from the mound.

The attacks long precede the final injury and death which is remote and may not follow vigorous attacks upon larger trees.

The same attacks are made upon small annuals and upon inanimate objects that could not cast shade upon the mound.

Attacks are made near the bases of trees and at the bases of leaves and near the tips of shoots, apparently with reference to the position of the part of the ant's journey rather than to any anatomical peculiarity of the part attacked compared with other parts.

It is puzzling that the instinctive reaction of these ants to enemies that move or have scent should sometimes be called out against things that seem to be attacked merely because they are on or near the mound. May it be that this instinctive action is sometimes called out through internal states associated with the movements of the ant in its journey to and from the mound?

Attacks upon trees might thus appear as aberrations, being normal acts but applied in apparently senseless ways upon some occasions, when the ants seem exploding with berserker force and release their tendencies to run amuck upon harmless objects that force the ants to change direction of running and also can not be picked up and carried to the mound.

Conclusions

1. The ant *Formica exsectoides* F., in the Eastern United States, kills small trees and plants in Maryland as well as in states to the north.

2. The damage by destruction of leaves, growing tips and the attacks on the bases of stems seems to lead to no immediate gain by supplying food or lodging. Eventually, however, such destruction may prolong the period in which the ants enjoy the sunshine before the trees overshade the mounds.

3. The damage done is chiefly in plantations of young trees or in clearings that are naturally growing with weeds and trees.

4. That the ant distinguishes between plants that may or may not shade the mound is not proved in Maryland.

5. Eventually, the forest may overshade the mounds despite the clearings which the ants tend to keep about

their mounds; hence the damage to the forest may be transitory in any one region.

6. While in plantations it may be expedient to exterminate the ant, it is to be questioned whether in natural forests the damage done is of sufficient magnitude to warrant efforts toward destruction of this ant.

7. The harm done by these ants in defending the insects that suck the juice from certain trees is partly offset by the good they do in killing other insects and it may be that further investigation of the part played by the ant in the economy of the forest will reveal reasons for regarding these ants as playing some useful part in the long-enduring sequence of vegetations.

8. What excites the ants to these attacks upon trees near the mound is a subject of speculation, but it may prove to be of the nature of instinctive action rather misdirected by unusual internal factors, yet becoming of use to the race of ant colonies.

A PROPOSED TECHNIQUE FOR THE INVESTIGATION OF RACIAL DIFFERENCES IN INTELLIGENCE

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THE following article is a digest outlining certain portions of the writer's Ph.D. thesis, presented at Harvard University in June, 1926. It represents an attempt to bring out the salient points of his proposed technique for investigating racial intelligence and to illustrate an application of these points. He has endeavored, in consideration of both the anthropological and psychological viewpoints, to outline a method of procedure which he hopes will be free from many of the objections levelled at previous investigations of this subject.

Whether or not this attempt has been successful is really immaterial. The writer merely wishes to stress a greater consideration for a more conservative point of view and to protest against the tendency of assuming certain differences to be innate which can possibly be explained on an environmental basis. He begs to thank the members of the faculty in the departments of education and anthropology at Harvard for their help and criticism.

I

We seriously need a technique of investigation which will yield incontrovertible results on the subject of racial differences in intelligence. Our present procedure is literally leading us around in a circle.

A certain small group of psychologists have carried on very extensive research on this subject. This has mostly been on various so-called "racial" groups in the United States, although of late the work is being carried farther afield. In the great majority of cases, the investigation

has been prosecuted by means of the verbal test, although here again we are finding an acute realization of the limitations of this device and a consequent swinging to the non-language test. This section of the psychological school is in the main committed to racial differences in intelligence.

Opposed to them, we have probably the majority of the psychological school and practically all the anthropologists who view their results and conclusions with very open suspicion.

The reason for this suspicion is obvious. The critics have very grave doubts whether we are able to measure the intelligence of any group of people with tests devised to gauge that of another group. The environment of the two may be entirely different, their social customs and traditions may vary widely, they may not even speak the same language or may speak it with different degrees of facility.

Moreover, even when we have tested these different groups we have not treated them from a "racial" standpoint. We have merely been testing a cultural group which may or may not be racially homogeneous. In the case of the Jap, for instance, it might be but probably is not. In the case of the Jew or Italian, there is even greater uncertainty. Race depends on physical characteristics only.

Under these circumstances, our obvious move is to search for a new technique which will yield results beyond controversy. To be sure, these results may be wholly negative, but what we wish is reliability—whether positive or negative conclusions follow is quite immaterial.

First let us consider our verbal tests. It seems reasonable to claim that they actually do measure certain mental abilities and that these abilities may, for our purposes, be termed intelligence. To be sure, different tests may not always measure the same abilities, but the same test probably measures much the same abilities at all times.

However, these verbal tests can really only yield valid results when used within a cultural group. As an example of a cultural group we would use the nation, provided there are no language handicaps or racial barriers within the nation. In this case, we would have to limit ourselves to these smaller groups. The Negro groups in the United States would be an example.

Again, if we are to get *racial* comparisons, we must relate our tests to physical characteristics, for these determine race, but we must be careful in our choice of these characteristics. They must be truly racial in that they are quite beyond the influence of environment.

The writer proposes that, in the case of our verbal tests, we must limit them to use within cultural groups, we must correlate them with physical criteria which are truly hereditary, which have a random distribution throughout the entire group and which do not subject the owner to any social discrimination at present and have not done so in the immediate past.

Thus he proposes that, if a correlation were found between color of hair or eye and intelligence within the North European group in this country, it would be due to an innate racial difference, since hair or eye color are normally distributed—probably—throughout this sample, do not subject the owner to racial discrimination, have not done so in the near past and are probably beyond the influence of immediate environment. The presence or absence of the epicanthic fold within the same group could be similarly used; also, perhaps the head indices.

It must be borne in mind that we can not correlate intelligence with physical characteristics which are subject to the influence of environment and hope for valid racial data. Thus stature may be influenced by environment, and the same factors in the environment which cause a greater stature may also result in a higher intelligence— we refer especially to social stratification—thus causing a correlation between intelligence and stature which may be in no way due to innate differences.

With this technique we would be able to say that within the North European group blue eyes, light hair or long head were correlated positively or negatively with, or wholly unrelated to, intelligence. We could probably carry the same technique over to the Jew samples in this country and from them back to the Jew samples in Europe whence they come.

But we can not mix our results. We can not compare gross scores of these groups. Nor can we say even that a difference of, say, three points I. Q. between blue eye and brown eye in the North European group is equal to the same in the Jew group. We can merely say that blue eye—or brown eye—is consistently ahead in both groups and leave it at that.

The non-language test presents a somewhat different problem, and yet the writer feels that it is virtually subject to the same restrictions with one very important reservation.

Merely because we have eliminated the language problem we have no guarantee that we are therefore entitled to use a test designed for American children on Italians, Australian natives or on Eskimaux. We have eliminated a factor which is obvious and which very evidently places the other groups who speak a different language at a serious disadvantage. That, however, is no guarantee that there are not still other factors, resulting from different environments, which, while not so obvious, are at least of considerable importance. The writer strongly protests against the assumption that we are entitled to compare gross scores arising from such procedure.

If we wish to compare gross scores we must confine ourselves to the same cultural groups and probably to the same social strata within those groups, although the latter point is debatable. We certainly must confine ourselves to the same cultural group, however, unless our results are to arouse a storm of criticism.

By using the technique required for the verbal test, we can correlate these results with individual physical cri-

teria within these groups, provided these criteria answer our requirements, *i.e.,* are strictly hereditary, have a random distribution throughout the entire group, do not subject the possessor to social discrimination and have not done so in the immediate past—say five hundred years.

Moreover, even though we are only speaking of single physical characteristics or very small groups of such, we may say that they are innate racial differences. Our interest lies in the relation between intelligence and physical criteria which are racial in character.

But we must bear in mind that this only applies to intelligence as tested by the test. This may not be the same intelligence which is tested by another test and need not be general intelligence. For our purposes here, intelligence must literally be what the tests test.

The non-language test has one advantage which is well-nigh inestimable. If properly constructed, we can use the same test on practically every cultural group—subject to definite limitations.

Let us take a practical illustration. Suppose the Knox cube test measures certain abilities in the white school child of the United States. This test is non-linguistic and needs no apparatus. Stones or sticks could be used as well as the cubes. Under these circumstances, are we justified in assuming that this test will measure the same abilities in the Eskimo or in the Hindu? In the opinion of the writer, we are so justified, although he sees no way of obtaining absolute proof. He will merely say that there is a strong probability that the test measures the same abilities in all cases, which, however, does not mean that we are justified in comparing gross scores.

Suppose, for instance, that we arrange a battery of tests—or a single comprehensive test—such as the Knox cube, which is non-linguistic and requires no apparatus, save of the very simplest nature. Let us suppose that the white child makes a gross score of 100, the Eskimo child of similar age and sex a score of 75, and the Hindu child a score of 50. We are not justified in comparing these

gross scores, in spite of the fact that the test is non-linguistic and requires no apparatus, for while the test may measure the same abilities we do not know the effect that different environments have had on these abilities.

However, let us suppose that on this same test the long-headed white child exceeded the broad-headed white child; in other words, that intelligence—so measured—gave a negative correlation with the cephalic index. Suppose that the same occurred in the case of the Eskimo child and the Hindu child and suppose that this tendency was consistent and significant.

This, I submit, is an entirely different matter. We would have demonstrated that long-headedness was related to the particular abilities tested in all three groups. The abilities thus measured are probably the same in these samples. We could thus say that, in this particular phase of intelligence, the long-headed child in these three groups exceeded the broad-headed child and that this difference was due to an innate difference in intelligence—a "racial" difference in the true sense of the word. Similarly, we could use the epicanthic fold, form of hair and indeed any truly anthropological criteria which lends itself to this technique.

We must bear in mind that even when dealing with such criteria we can not lump our scores of these different groups and express our relationship in a single correlation coefficient. We can merely express the relationship for each cultural group. The scores of these different groups are not comparable, except possibly on the basis of rank order.

Finally, this technique demands exact anthropometric methods. The criteria which lend themselves are just those which do not make for a consciousness of kind, are often difficult to detect and require skill and practice in their exact description.

II

In order to give a concrete demonstration of his contentions the writer will draw on certain sections of his

Ph.D. thesis, presented at Harvard in June, 1926. This work, entitled "Racial Factors in Intelligence," deals with fairly large samples of North European, Italian and Jew children drawn from the towns of Medford and Revere, Mass. The data were collected by the Harvard Growth Study, under the direction of Professor W. F. Dearborn. Practically all the first-grade pupils in these towns were given certain physical and mental measurements in the year 1922–23. These measurements have since been repeated at yearly intervals, but with some modifications.

For purposes of illustration we will confine our attention to a very small section of the material dealt with in the thesis. We will take the three head measurements and their derived indices and will consider the relation between these data and the Dearborn Group Test of Intelligence, Form A, the Otis Group Intelligence Scale, Primary Examination and the Stanford Revision of the Binet-Simon Test. We will deal only with the physical measurements as taken in the school year 1923–24 and with the results of the Dearborn A as given that same year, the Otis the year following and the Binet given at various times—mostly the same year. We will further confine our attention to the children of North European extraction who were in Grade I and who were in their sixth year when the testing program started in 1922–23. Their presence in the North European group is dependent on ancestry. Here are included children both of whose parents are of the old American stock as well as later immigrants from the British Isles, from Scandinavia, from the Baltic States and from English-speaking Canada.

These children were thus in their seventh year when the physical measurements were taken and the Dearborn A given, in their eighth when the Otis was administered and in their sixth, seventh or eighth on receiving the Binet.

We thus have the several conditions fulfilled, at least in theory, which we have demanded in order to get a valid test of racial differences in intelligence.

First we are confining the use of our intelligence tests to one cultural group. Within this group there are no language handicaps. To be sure there are obvious differences in social stratification, but these are of no account with our present technique. This will be more fully explained later.

For the purpose of argument we are *supposing* that shape of head is truly hereditary in nature, that it has a random distribution as to social strata throughout the entire group in question and that it subjects the members of this group to no social discrimination and has not done so in the immediate past. Needless to say, the first supposition is an open question and the second would require very careful checking. We are here interested in demonstrating a technique and are making these assumptions purely with this end in view.

The sexes, of course, must be kept separate. Also age, with its possible relation to shape of head, must be partialled out or kept constant—here kept constant. Should stature be related to intelligence on the one hand and head shape on the other, and should our groups be spread through a large stature range, it also would have to be allowed for. However, in our present seven-year-old sample the errors introduced by this factor would be wholly insignificant.

If, then, we adopt the head indices as our criteria of race—Dixon does so to a large degree—are we not entitled to say that any correlation between head shape and intelligence within this six-year North European group would be a truly racial difference in intelligence? Of course we would have to consider the reliability of the difference and also our definition of intelligence. But the differences, subject to such restrictions, would probably be regarded as truly racial.

Again we have one very great advantage. We are entitled to completely overlook the problem of social stratification, because our racial criteria are particularly chosen so as to have no correlation with economic position. High cephalic index is, theoretically, found just as frequently in the lower social orders of this North European group as in the higher and the same applies to the other head indices.

Let us now consider the relation between the various head indices and results on the three intelligence tests in this group of seven-year-old North European boys and girls. See Table No. 1.

TABLE No. 1

CORRELATIONS BETWEEN THE VARIOUS HEAD INDICES AND THE DEARBORN GROUP TEST OF INTELLIGENCE, FORM A, THE OTIS GROUP INTELLIGENCE TEST, PRIMARY EXAMINATION AND THE STANFORD REVISION OF THE BINET-SIMON TEST IN A GROUP OF CHILDREN OF NORTH EUROPEAN EXTRACTION DRAWN FROM MEDFORD AND REVERE, MASS. THE CHILDREN WERE IN THEIR SEVENTH YEAR WHEN THE HEAD MEASUREMENTS WERE TAKEN.

	MALE		
	Cephalic Index	Length-Ht. Index	Br.-Ht. Index
Dear. A.	+.11 ± .08	+.07 ± .08	+.01 ± .08
Otis	+.06 ± .05	+.03 ± .05	−.02 ± .05
Binet	+.06 ± .07	+.15 ± .07	+.06 ± .07
	FEMALE		
	Cephalic Index	Length-Ht. Index	Br.-Ht. Index
Dear. A	−.12 ± .06	−.01 ± .06	+.03 ± .06
Otis	−.07 ± .05	−.09 ± .05	−.06 ± .05
Binet	−.02 ± .06	−.11 ± .05	−.13 ± .05

Here we see quite clearly that these correlations, expressed in terms of the Pearson "r," have no significance. In other words, if we use the head indices as criteria of race under circumstances in which language handicap, racial prejudice and social stratification can not enter, we obtain no correlation between race and intelligence, as measured by these three tests.

And yet I feel that this method of attack is perfectly legitimate, certainly much more so than our usual pro-

cedure. The head indices are possibly the best racial criteria that exist. We are certainly much nearer the anthropological conception of the term when we apply "race" on this basis rather than on the basis of nationality. It is in this later sense that we have almost exclusively used it up to the present.

Let us now demonstrate the necessity of properly choosing our racial criteria. In Table No. 2 we have the same group of children under consideration. Here, however, we are correlating our intelligence tests with the *gross* head measurements, not with the indices.

TABLE No. 2

THE INTELLIGENCE TESTS ARE HERE CORRELATED WITH THE GROSS HEAD MEASUREMENTS. THE GROUP UNDER CONSIDERATION IS THE SAME AS IN TABLE 1

	MALE		
	Cephalic Index	Length-Ht. Index	Br.-Ht. Index
Dear. A	+.08 ± .08	+.18 ± .08	+.17 ± .08
Otis	+.20 ± .05	+.25 ± .05	+.15 ± .05
Binet	+.15 ± .07	+.23 ± .06	+.21 ± .06
	FEMALE		
	Cephalic Index	Length-Ht. Index	Br.-Ht. Index
Dear. A	+.16 ± .06	+.01 ± .06	+.03 ± .06
Otis	+.19 ± .04	+.10 ± .05	+.02 ± .05
Binet	+.14 ± .05	÷.14 ± .05	+.04 ± .06

Here our correlations are in some cases four and even five times the probable errors. Yet these relations are of very little value from a racial point of view. A large head goes with large stature. Large stature is found, even among these children, in the higher social strata, and may here be caused by better nutrition. If so it can not be used as a racial criterion. Moreover, educational opportunities are far better in this section of society, and all intelligence tests tend to be influenced in some degree by schooling. In other words, the gross head measurements, being possibly dependent on cultural conditions

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Dixon's[1] criteria of race—head and nasal indices—would probably lend themselves admirably to such a treatment, and if, say long high head and narrow nose always demonstrated their superiority over all other types, we might have some real justification for our Nordic myth.

One thing is certain, our present methods of investigating racial differences in intelligence can not command a unanimity of opinion. There are too many obvious flaws. The writer advances this technique as a tentative proposal toward rectifying some of these. Definition of race in a strictly anthropological sense of the word, the use of racial criteria which are truly hereditary and quite free from social stigma, and the careful correlating of intelligence tests with these should yield valid results. However, the problem is one of great complexity and the writer certainly does not claim to have solved it—he has merely registered a protest and advanced a very tentative mode of attack.

[1] R. B. Dixon, ''Racial History of Man.''

SHORTER ARTICLES AND DISCUSSION

NON-INHERITANCE OF THE TEMPERATURE EFFECT ON BAR EYE IN DROSOPHILA MELANOGASTER

THE size of the eye in Drosophila is dependent upon several known hereditary and environmental factors. The effect of these factors is a striking one and may be readily measured by the ommatidial or eye facet counts. An environmental factor may exactly balance a hereditary one so that the results are indistinguishable. When individuals of the bar eye stock which were used in the present experiments are raised at 27° centigrade they resemble individuals raised at 17° which have changed in hereditary constitution from bar to ultra-bar. In other words, the somatic effect of a change in hereditary constitution from bar to ultra-bar has a temperature equivalent of approximately ten degrees centigrade. Flies differing in hereditary constitution but raised at the same temperature may be arranged according to eye size in a series which duplicates, within limits one obtained with flies of a particular hereditary constitution raised at different temperatures. Examples of approximate facet values of females of different hereditary constitutions at 27° are:

Round eye	800
Emarginate	300
High selected Bar	150
Unselected Bar	80
Low selected Bar	40
Ultra-bar	22

Examples of facet values of females of the same hereditary constitution but raised at different temperatures are:

Low selected Bar at 17°	100 facets
Low selected Bar at 27°	40 facets

The material seems to furnish ideal conditions for the study of a possible direct effect of temperature upon the germ cells or of a possible transfer of the somatic temperature-effect to the

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germ cells and thus for testing under most favorable circumstances the hypothesis of inheritance of somatic effects induced by environmental change.

A general observation of the bar-eye stocks over a period of ten years led to the conclusion that there is no marked inheritance of the temperature effect. It seemed desirable however to devise a direct test because no critical experiments for this special purpose has been made. There was need especially of testing the possibility that there may be a slight effect in each generation which is cumulative and noticeable after a considerable number of generations.

The present experiment was started in September, 1922, for this purpose. Its general plan was to take an inbred selected stock with a known history and from a single pair of such a stock to keep some of the offspring at 17° and others at 27°. At the lower temperature the size of the eye and the corresponding number of eye facets is about two and a half times as great as at the highest temperature. Tests for change in hereditary constitution were made at intervals by reciprocal transfers of some of the individuals from one temperature to the other. At each test there was thus a comparison of flies raised continuously at one temperature with flies raised at the same temperature for one generation whose ancestors had been kept for many generations at the higher (or lower) temperature.

In February, 1923, after the flies had been at the two separate temperatures for five months the 27 degree line was in the eleventh generation and the 17 degree line in the fifth generation. Twenty single pair matings of the 27 degree line were put at 17 degrees in the same incubator with the continuous 17 degree line. Correspondingly twenty single pair matings of the 17 degree line were put at 27 degrees in the same incubator with the continuous 27 degree line. Two reciprocal comparisons may therefore be made. On the one hand, the flies whose history is five continuous generations at 17 degrees were compared with those whose history is eleven generations at 27 degrees followed by one generation at 17 degrees. On the other hand flies whose history is eleven continuous generations at 27 degrees were compared with the flies whose history is five generations at 17 degrees followed by one at 27 degrees.

Similar tests were made after thirteen months when the 27 degree line was in the twenty-sixth generation and the 17 degree

line in the eleventh; after 18 months when the 27 degree line was in the 38th generation and the 17 degree in the 16th generation, and, after 30 months when the 27 degree line was in the 64th generation and the 17 degree in its 26th.

The results are expressed in temperature equivalents, i. e., in temperature differences which would produce corresponding facet number changes.

On the hypothesis of inheritance of the temperature effect flies raised at 17 degrees whose ancestors had been kept at 27 degrees should have smaller eyes than those with a continuous 17 degree ancestor. Similarly flies raised at 27 degrees whose ancestors had been kept at 17 degrees should have larger eyes than those with a continuous 27 degree ancestry.

In the data agreement with the hypothesis is expressed as positive and disagreement as negative.

Length of treatment	Males at 17	Males at 27
5 months	$+0.14 \pm 0.23$	$+0.25 \pm 0.$
13 months	-0.06 ± 0.19	$+0.22 \pm 0.$
18 months	-0.22 ± 0.15	$+0.80 \pm 0.$
30 months	-0.11 ± 0.15	-0.18 ± 0.21

Only one of these values, that at eighteen months in males at 27 degrees, can be considered as significant. It is to be noted, however, that in the continuation of the experiment this condition is not maintained for after thirty months the value is negative though not significantly so.

In the light of these results it is highly improbable that there has been any significant inheritance of the temperature effect during the recorded period. The experiment is being continued.

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A POSSIBLE EXPLANATION FOR WINGE'S FINDINGS IN *LEBISTES RETICULATUS*

IN a recently published paper, Winge (1927) described nine new genes in *Lebistes reticulatus*. These nine genes, together with those previously described, make a total of eighteen genes which have been genetically investigated in this species of fish.

All of them are dominant, and all are sex-limited; that is, they appear or produce visible effects in males only. (In a few instances, however, they were detected in old females; but these females showed signs of a change towards maleness.) Seventeen of these genes were found to be sex-linked, and one was located in one of the twenty-two pairs of autosomes. Nine of the sex-linked genes were located in the Y-chromosome, three were in the X-chromosome, and the remaining five showed a few instances of crossing-over between the X- and Y-chromosomes. Crossing-over, however, was so rare that it could be considered as an exceptional rather than a regular phenomenon.

The results obtained by Winge can not be questioned. The data given by him for the behavior of the sex-linked characters indicate plainly a linkage between these characters and the X- or Y-chromosomes. However, a question as to the validity of the interpretation of the data arises when one considers the probability of seventeen out of eighteen dominant factors being located in the sex-chromosomes. Winge (1922) found that the *Lebistes reticulatus* has twenty-three pairs of chromosomes, one pair of them being sex-chromosomes. All the chromosomes were pictured as being similar in size. Now a dominant mutation can equally well be detected if it occurs in the sex-chromosome or in any of the autosomes. If it is assumed that there is no difference in mutability between the different chromosomes, then it would be expected that the eighteen dominant genes would be equally distributed among the different chromosomes. As already mentioned, almost the opposite was found.

If it is assumed that the chances of a dominant mutation arising in any of the chromosomes are equal, then the probability of seventeen out of eighteen mutations being located in one out of twenty-three chromosomes is one in about 8 times 10^{21}. This figure is so low that the case could not be considered as possible. The observed results might be accounted for by the assumption that the mutability of sex-chromosomes is higher than the mutability of the autosomes. It would be necessary, however, for the sex-chromosome to be three hundred and seventy-four times as mutable as the autosomes to make the chances of seventeen out of eighteen mutants being sex-linked equal. Such a difference in the mutability is not impossible, but neither is it probable, and there is nothing in other genetic work to support it. Nor is there much probability in the supposition that mutations affecting the

color of the body would occur more frequently in the sex-chromosomes than in the autosomes.

The mutants described by Winge did not originate in his cultures, but were acquired with the fishes obtained from different breeders. The question arises if the methods of handling the fishes used by breeders would tend to eliminate autosomal mutant characters and preserve sex-linked ones. If mutants desirable to the breeders have an equal chance of occurring in any of the chromosomes, then dominant autosomal mutants have a higher chance of being preserved than dominant sex-linked mutants (assuming that all of them are sex-limited and show in the males only). The mutants occurring in the Y-chromosomes would be transmitted to all the male progeny of a mutant male, and so these mutants do not have much chance of being lost during the breeding. The mutants, however, occurring in the X-chromosome may easily be lost in the breeding. They would not appear in the first generation of a cross between a female and a mutant male until the inbreeding has been carried so far that the females used in the breeding become heterozygous or homozygous for the character. The autosomal mutants would have almost as much chance of being preserved as the Y-chromosome mutants. In the case of an autosomal mutant about half of the male progeny of a mutant male would show the character.

Is there any other explanation than that given by Winge which would fit the observed facts better? The difficulties encountered in Winge's explanation could be avoided if it assumed that the chromosomes of Lebistes do not segregate independently, but certain ones of them always go together at the reduction divisions. If that assumption is made, then characters located in the chromosomes which go together with the X-chromosome would appear to be located in the X-chromosome. The sex-linkage effect, then, would not necessarily be a result of the location of a character in the sex-chromosomes, but might also be a result of an association between the chromosomes. The evidence given by Winge indicates the presence of at least two chromosomal complexes in Lebistes. One of them, the large one, involves the sex-chromosomes and the majority of the autosomes. The observed recombinations between factors could be accounted for by breakages in the chromosome-complex just as well as by crossing-over between homologous chromosomes.

The assumption of this association between the chromosomes would not only account for the high number of sex-linked mutants,

but would also explain the lack of recessive color characters in Lebistes. Any recessive sex-linked character which would show in the males only (as is the case with all characters so far described in Lebistes) would have an exceedingly low chance of being detected. For such a character to be visible the mutation would have to occur in both of the homologous chromosomes, or would have to be transmitted to the other set of homologous chromosomes by breakage in the chromosomal-complex. The autosomal recessives would have as good a chance to show up as the autosomal recessives of any other organism. Since, apparently, the autosomal chromosome-complex involves very few autosomes, the chances for these mutations occurring and being detected are low.

There are several cases described in literature which indicate association between chromosomes. Cleland (1926) assumed that, at the reduction division in the pollen-mother-cells of *Oenothera biennis*, all maternal chromosomes went to one pole and all the paternal to the other pole. Thus only two chromosome-complexes were formed, which were the same as the complexes which united in the first place to form the plant. According to Schrader (1921, 1923), in at least three species of Pseudococcus, no synapsis occurs in the males. In the spermatocyte divisions, chromosomes are grouped into two groups, five in each. The groups go to different poles at the reduction division. The facts indicate that the two groups are composed of paternal and maternal sets of chromosomes, respectively. Metz, Moses and Hoppe (1926) described the lack of synapsis and the budding-off of one set of chromosomes at the spermatocyte divisions of males in four species of Sciara. The genetic evidence obtained in two species (Metz, 1927) indicated that the male set of chromosomes is thrown out and the female set retained. A lack of synapsis in Lebistes (Winge, 1923) suggests the possibility of chromosomal abnormalities, and gives a support to the view that the high number of sex-linked characters found by Winge might be due to association between the chromosomes.

SUMMARY

In *Lebistes reticulatus* Winge described eighteen genes, seventeen of which are sex-linked, and only one autosomal. All of them are dominant. They affect the color of the body and are limited to males. Lebistes has twenty-three pairs of chromosomes, which are morphologically alike.

It has been shown that the chances of seventeen out of eighteen mutants being located in one of the twenty-three pairs of chromosomes are small. It is not likely that the difference in the mutability between the sex-chromosomes and autosomes could be so great as to account for the observed facts. It is also not probable that the mutants affecting body color would be more likely to occur in the sex-chromosome than in the autosomes. Neither is it probable that the breeders from whom the mutants were obtained would select in favor of sex-linked mutants.

A suggestion is made that the high number of sex-linked mutants in Lebistes might be accounted for by the assumption of association between chromosomes (attachment of non-homologous chromosomes).

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PRELIMINARY NOTE ON THE GENETICS OF WAX IN PISUM

Introduction

In an earlier contribution[1] the wax character in peas was considered to be determined by the action of two genes, a basic one, Bl, and a second factor, W. Bl gives little wax in itself, while W together with Bl gives much wax (''glaucous''), but is inactive when present alone. In the same paper it was surmised

[1] S. J. Wellensiek. ''Pisum-Crosses'' II. *Genetica* 7: 337–364. 1925.

that the character in question probably is influenced by more than two genes. New data are at hand now, and these make it evident that the wax-character in its genetic nature is much more complicated indeed than was thought before.

THE PARENTAL TYPES

A number of emerald varieties, together with the glaucous type, were divided into five classes according to the amount of wax which developed on the stems.   Three classes were: much, little, and nothing, while in between much and little and in

TABLE 1

THE FIVE TYPES OF PARENTAL REACTION

Type		Amount of wax
4	....	Much (normal ''glaucous'' type)
3	....	Between little and much
2	....	Little
1	....	Between little and nothing
0	....	None (extreme ''emerald'' type)

TABLE 2

RESULTS FROM CROSSING

Combination of parent types	F_1	Type of F_2 segregation	Number of F_2 plants	Year in which F_2 was studied
4 × 4	4	No segregation	Several thousands of plants in several crosses	1922–1927
4 × 3	4	3:1	1045	1927
4 × 2[a]	4	3:1	561	1927
4 × 2[b]	4	3:1	994	1922,[2] 1923,[2] 1927
4 × 1	4	3:1	252	1927
4 × 0	4	3:1	305	1927
3 × 3	3	No segregation	515	1927
3 × 2[a]	4	9:3:4	243	1925
2[a] × 2[b]	4	9:7	1124	1927
2[b] × 2[b]	2	No segregation	464	1927
2[a] × 1	2	3:1	455	1927
2[b] × 1	4	9:3:4	223	1925
2[b] × 0	4	9:3:4	bog	1925,[2] 1926

[2] Published before (*Genetica* 7, 1925; see pp. 12–14 and pp. 339–341).

between little and nothing two more types occurred. For convenience's sake, the five classes were indicated as 4, 3, 2, 1 and 0, as shown in Table 1.

These types are usually very sharply distinguishable and no classification difficulties were met with. Two genetically different types 2 were found, indicated in the following paragraph as 2^a and 2^b.

Results from Crossing

In Table 2 the results of the crosses between several of the types are summarized.

Interpretation

The above-mentioned experimental results have led to the hypothesis that three interacting sets of factors are influencing the wax character. One of these sets consists of two allelomorphs, the other two of three. If we name the factors $Bl - bl$, $W_2^a - W_1^a - w^a$ and $W_2^b - W_1^b - w^b$, the following genetic formulae account for the experimental results:

$$Bl\ Bl\ W_2^a\ W_2^a\ W_2^b\ W_2^b:\ \text{type 4, much wax}$$
$$Bl\ Bl\ W_2^a\ W_2^a\ W_1^b\ W_1^b:\ \text{type 3, between much and little wax}$$
$$Bl\ Bl\ W_2^a\ W_2^a\ w^b\ \ w^b\ :\ \text{type } 2^b,\ \text{little wax}$$
$$Bl\ Bl\ W_1^a\ W_1^a\ W_2^b\ W_2^b:\ \text{type } 2^a,\ \text{little wax}$$
$$Bl\ Bl\ w^a\ \ w^a\ \ W_2^b\ W_2^b:\ \text{type 1, between little and no wax}$$
$$bl\ bl\ W_2^a\ W_2^a\ W_2^b\ W_2^b:\ \text{type 0, no wax}$$

Consequently, Bl is basic factor for W_2^a or W_1^a, while Bl and W_2^a or Bl and W_1^a together are basic factor for W_2^b or W_1^b. Furthermore, W_2^b and W_1^b evidently have a weaker effect than W_2^a and W_1^a. The F's of all possible combinations between the parental types, not mentioned in Table 2, were studied in 1927 and confirm the above hypothesis. It is remarkable that all emerald types differ from the glaucous by one factor. This suggests the possibility that in each case they have arisen by mutation of one factor from the glaucous type, which is probably older phylogenetically.

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